NUTRITIONAL MARKERS IN HYPERALIMENTATION AND PROTEIN SPARING THERAPY

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ABSTRACT

This thesis is concerned with certain aspects of nutrition as related to patients in hospital. An assessment was first made of one protein, transferrin, to determine whether it is of the same value as an index of nutritional restoration in non-stressed, malnourished adult patients as it is in children. The change in serum concentration was compared to that of albumin over a two week period in groups given either intravenous hyperalimentation, or enteral nutrition. The energy relationships of these methods of nutrition were also calculated.

For enteral feeding there was a mean increase in transferrin of 64 mg/dl from an initial mean concentration of 152 mg/dl and for intravenous hyperalimentation the increase was 68 mg/dl from 138 mg/dl. In contrast there was no improvement in the serum albumin concentration over the same period of time. Transferrin can therefore be considered a sensitive marker of early nutritional repletion.

Effective therapy was delivered at a calorie intake of 33 Kcals/kg by the enteral route and 42 Kcals/kg by the parenteral route. At equivalent levels of energy intake the dietary protein utilisation was consistantly greater with enteral feeding. It was concluded that enteral feeding is a more efficient method than intravenous hyperalimentation of using available energy for protein restoration.

A further study was carried out to examine the proposition that

in protein sparing therapy after surgery the metabolic adaptations to the use of fat as an energy source permit amino acids to be made available to the viscera, particularly the liver, for protein synthesis. Three intravenous regimens of low calorie content were used to assess the role of albumin, transferrin and prealbumin, the acute phase reactant proteins and the immunoglobulins as possible nutritional markers after major abdominal surgery. The response to traditional dextrose infusions was compared to that with amino acid infusions and also to pre-operative partial ketoadaptation (achieved by a similar carbohydrate-free protein diet) continued in the post-operative period with amino acid infusions.

Both groups who received protein sparing therapy with amino acids had improved nitrogen balance. Although the group partially ketoadapted before surgery held its concentration of albumin, transferrin and prealbumin better than the other two groups over the first week following surgery, no difference was noted between the groups receiving dextrose or amino acids, despite a much improved nitrogen balance in the latter group ($p < 0.001$).

None of the individual acute phase reactant proteins nor the immunoglobulins were found to be of any value as indices of nutritional support. However, the pattern of response in the patients partially ketoadapted prior to surgery was noted to be different in that there was much less of a variation, either as a rise or as a fall, from the pre-operative concentration than in the other groups.

The metabolic changes noted with groups receiving protein sparing therapy are in agreement with the concept of the mobilisation of fat as an energy source, and of this therapy as supportive to hepatic protein synthesis. It is suggested that this may be due to the action of ketone bodies on skeletal muscle, possibly related to their effect on branched chain amino acid oxidation.

PREFACE

In the United Kingdom over the past four years it has been the custom to report measurements in S.I. units. This is not as yet a worldwide convention, and in the United States S.I. units are not normally required for medical publications. I have elected not to convert all measurements into S.I. units in this thesis for the following reasons. The analytical work was carried out in four different laboratories in the United States and their results are presented in the units recorded. Secondly, work which has been carried out in this country, for example the study by Young, Collins and Hill from Leeds - Reference 165, and which is published in specialist journals is not converted to S.I. units. The third and most important reason for adhering to conventional units is that comparisons with other reported studies are very much easier and less confusing if the units are the same. As much of the literature on one aspect of this thesis, protein sparing therapy, has originated from various centres in the United States, I felt it justifiable to continue to use conventional units.

The conversion table on the next page gives the appropriate conversion factors to S.I. units.

CONVERSION TABLE

Blood Constituents	Units Used	Conversion Factor (multiply by)	S.I. Unit
Albumin	g/dl	10	g/l
Acute Phase Proteins	mg/dl	0.01	g/l
Free Fatty Acids	mEq/l	1	mmol/l
Glucose	mmol/l (mM)	1	mmol/l
Immunoglobulins	mg/dl	0.01	g/l
Insulin	µUnit/ml	1	µUnits/dl
Ketone Bodies	mmol/l (mM)	1	mmol/l
Lactate	mmol/l (mM)	1	mmol/l
Prealbumin	mg/dl	0.01	g/l
Transferrin	mg/dl	0.01	g/l
Dietary Intake			
Energy	Kcalories	4.19	KJ
Nitrogen	g/day	70	mmol/24 hr.

INTRODUCTION

Over the past few years there has been increasing attention paid to nutritional needs of surgical patients. An objective assessment of nutritional status is a necessary requisite for the delivery of appropriate nutritional therapy, particularly as such therapy is costly. Mild malnutrition in a patient often is unrecognised and a prolonged period of protein deficient semi-starvation following surgery may change the clinical status to one in which severe limitations in terms of response to infection and ability to heal wound are imposed on the patient.

Moderate and severe malnutrition can be identified by a number of investigations which include accurate anthropometric measurements of the various compartments of the body, biochemical tests and skin testing for delayed hypersensitivity (1). The triceps skin fold quantitates fat reserves. Skeletal muscle is proportional to the mid-upper arm muscle circumference. The urinary creatinine-height index gives a useful approximation of the lean body mass as long as renal function is stable. The serum or plasma concentrations of albumin and transferrin, proteins secreted by the liver, and skin testing for delayed hypersensitivity reactions with such recall antigens as Candida, Mumps and Streptokinase-Streptodornase, indices of immune function, give useful information about aspects of visceral metabolism.

By the use of these parameters a nutritional profile can be drawn up and any patient placed in a broad classification of normally nourished, mildly, moderately or severely malnourished. This

profile and classification is helpful in planning a suitable nutritional regimen if such is required. The same parameters are, of course, useful in measuring the objective response to nutritional therapy.

Using these techniques in an American urban general hospital the incidence of moderate or severe malnutrition in surgical patients was shown to be around 50% (2). A proportion of the patients were considered to have developed malnutrition during their hospital stay, a finding which Bistrian and his colleagues ascribed to prolonged inattention to nutrition in stressed, catabolic patients (3). A similar finding of "unrecognised" malnutrition in a high proportion of surgical patients in a similar type of hospital in the United Kingdom (4) illustrates how little attention has traditionally been paid to this aspect of surgical care, even in communities in which pre-existing nutritional deprivation is not normally considered to exist.

ALBUMIN - A TRADITIONAL NUTRITIONAL MARKER

Albumin has served as the prime indicator of protein-calorie malnutrition in both medical and surgical patients for a long time (5). It is the major circulating protein in the body with the roles of maintaining colloid osmotic pressure and transport of metals, ions, hormones, drugs and other metabolites. Extensive studies using radioactive iodine, and carbon labels have helped to identify the site of synthesis, and the method and rate of degradation (6). In any method by which the synthetic rate of a protein is measured there may be considerable technical difficulties. For example in the measurement of albumin synthesis using

iodine isotopes, there is the risk of denaturing the protein during isolation and labelling. There are also problems in calculation. As albumin is widely distributed throughout the body, and synthesis and degradation occur at the same time, the assumption must be made in calculating the synthetic rate that a steady state exists with respect to albumin metabolism. By another technique arginine, labelled with a carbon isotope, is incorporated into albumin in the liver which enables direct albumin synthesis to be measured (6). This method also requires certain suppositions to be made. Urea is split off during the incorporation of arginine and therefore it has to be assumed that the synthetic rates of urea and albumin are stable during the period of measurement and that urea and albumin are synthesised from the same arginine pool with urea being synthesised only from this one pathway.

It is not proposed to discuss detailed methods of albumin synthesis and give a critique of the technical problems or assumptions made nor indeed for any of the other plasma proteins mentioned in this thesis, but rather opportunity is taken to point out that difficulties exist both in measurement and the interpretation of data.

Albumin is synthesised only in the liver. It equilibrates between the intravascular and extravascular compartments such that 31 - 42% of the exchangeable albumin pool is in the plasma. Partial equilibration of an injected dose is rapid although ultimate equilibration to tissue extravascular spaces may take from seven to ten days to complete. The half-life of albumin is 14 - 19 days (6, 7).

During prolonged fasting, albumin synthesis is depressed. The catabolic rate does not, however, decrease immediately after protein deprivation, but only after there has been a drop in the plasma concentration of albumin.(8). Although the factors involved and site of catabolism are poorly defined, Kirsch et al have described separate independent control mechanisms for synthesis and catabolism (9). Refeeding leads to rapid stimulation of synthesis which is very sensitive to dietary amino acid supply (10), particularly that of tryptophan (11, 12) and possibly the branched chain amino acids (13, 14). These, under suitable hormonal control, appear to influence hepatic free and endoplasmic reticulum bound polyribosomal aggregations which enhance intracellular enzyme and secretory protein synthesis (7). Release of albumin into the extra-vascular pool leads to an increase in the plasma through equilibration between the intra and extra-vascular compartments. Howeveras the extra-vascular/ intra-vascular ratio is reduced in malnutrition and refeeding leads to fluid shifts and to an expansion of the extra-vascular space, the plasma concentration may not rise rapidly.

In stressful situations, either from surgical or other trauma, myocardial infarction of infection, serum albumin concentrations decrease (8, 15). The probable explanation is a redistribution between the intra-vascular and extra-vascular spaces. The proportion in the extra-vascular compartment increases due to wound oedema, reduced lymphatic return and sodium retention, all of which tend to expand this compartment. A further factor may be the increased permeability of gut mucosa through which there may be loss of albumin. Albumin may also act as a "feeder" protein,

being broken down into amino acids which are needed for many of the pathways which are stimulated by a stressful event (16). Acute phase protein synthesis, wound healing erythropoiesis and maintenance of immune function are but a few which require new proteins to be synthesised and hence amino acids to be available. The length of time taken to rise to normal serum concentrations makes albumin an imprecise "marker" of nutritional restoration.

A further practical point which should be made is that the practice of infusing albumin to restore low serum concentrations or in the management of hypovolaemic shock, which is ever increasing in hospitals, not only invalidates the use of albumin as a nutritional marker in those patients, but this has also led to escalating costs and a crisis of supply (7).

OTHER MARKERS OF EARLY NUTRITIONAL REPLETION

Recent studies, in which certain plasma proteins were monitored during nutritional therapy in children with protein-calorie malnutrition, have shown albumin to be a relatively insensitive indicator of early improvement (17, 18, 19, 20). Other proteins synthesised in the liver, of much shorter half-life were found to be more sensitive markers. Transferrin, with a half-life of seven to eight days was of intermediate sensitivity. Prealbumin (PA), with a half-life of two days and a retinol-binding protein (RBP), which has a half-life of only 12 hours, were shown to be more accurate markers of the early restoration of visceral protein status (17).

Transferrin , the main iron-binding protein, is a globulin which

is largely synthesised in the liver although peripheral lymphocytes (21), gonadal cells and the submaxillary glands may also contribute small amounts (22). It is neither taken up nor used in appreciable quantities by receptor tissues, nor plays any enzymatic nor metabolic role and as such can be considered a true carrier protein (23). There is equal distribution of transferrin between extra-vascular and intra-vascular spaces, a process which takes four to five days to complete.

Synthesis of transferrin is not as sensitive to dietary amino acid supply as albumin (10). In iron deficiency synthesis of transferrin is increased. One could expect that the co-existence of iron and protein deficiency would tend to produce a higher level of transferrin than a similar degree of protein deficiency alone. However, this information is difficult to find, as in studies in malnourished children there are also varying degrees of iron deficiency anaemia. In one study protein availability appeared to be the limiting factor in transferrin synthesis in children with only mild anaemia (24).

Prealbumin is also known as thyroid-binding prealbumin. This name indicates one of its roles, that of binding and transporting thyroxine. Although the greatest proportion of circulating thyroxine is bound to thyroid-binding globulin, some 15% in man is bound to prealbumin and a very small proportion to albumin. However, of the total plasma prealbumin probably less than 1% circulates in a complex with thyroxine (25). A very much smaller proportion of tri-iodothyronine is bound to prealbumin (26).

A more major role for prealbumin is in the vitamin A transport system in which there is a 1:1:1 molar relationship between retinol, which is vitamin A alcohol, retinol-binding protein and prealbumin (25, 27). Although some 40% of prealbumin molecules circulate in the plasma complexed with retinol-binding protein (28), the interactions of prealbumin with thyroxine and those with retinol-binding protein would appear to be controlled independently (26). The finding that the structure of prealbumin is a tetramer, and the postulate that each of the isomers contains a binding site for retinol-binding protein, is not borne out by available evidence (29). The molar relationship between prealbumin and retinol-binding protein remains constant in many disease states except chronic renal disease in which there are elevated serum retinol-binding protein concentrations but normal prealbumin concentrations (29). This would suggest that catabolism of retinol-binding protein takes place in the kidney and that, teleologically, the size of the complex (MW 72,000 with prealbumin 55,000 and retinol-binding protein 17,000) may be sufficient to prevent excessive renal loss of the smaller retinol-binding protein molecule and hence of vitamin A.

The relevance of prealbumin in this thesis is an index of nutritional repletion. In Thai children with protein-calorie malnutrition the rapidity of restoration of the serum concentration of both these proteins of the retinol transport system over that of albumin suggests that this system is resynthesised with a high priority (19). Significant increases in all compartments of the transport system were observed by the end of the week used to stabilise dietary intake before feeding specific protein

regimens, whereas there was no increase in albumin over the same period.

Further reported studies in Sengalese (17, 20, 26), Egyptian (30) and Central American (31) children parallel that reported in the Thai children. All of these confirmed the early parallel rise of serum prealbumin and retinol binding protein and showed highly significant correlations between these two proteins and vitamin A. The rise of vitamin A was considered to represent mobilisation from stores as a consequence of protein and calorie refeeding (30). However, in mild protein-calorie malnutrition supplementation with dietary vitamin A alone was sufficient to stimulate the liver to increase synthesis of the retinol transport proteins. It is suggested that secretion of retinol binding protein rather than synthesis may be compromised in vitamin A deficient states (31).

Although vitamin A supplementation may play a role in stimulating the release of these transport proteins, it is not proposed to discuss vitamin A metabolism further in this thesis. Not only are there considerable stores in the liver which may last for many months or years with no dietary intake (32), but most, if not all, patients in the study were given regular daily or weekly supplementation of vitamin A. Deficiency was not therefore considered to exist. Because of the parallel response and molar relationship of the two proteins, it was decided to carry out assays of only one, prealbumin, in one of the studies to be reported later.

A further point should be made about prealbumin. It has a high content of tryptophan and hence deficiency of this amino acid or a disordered metabolism have been suggested as causes for failure of synthesis of prealbumin (18). Restoration of tryptophan would therefore appear to play a double role in prealbumin, both in a non-specific way for hepatic synthesis and also as a necessary component of the protein.

DOES SURGICAL PRACTICE PRODUCE MALNUTRITION?

It is only by the use of appropriate criteria that the objective assessment of any treatment can be made. Anthropometric measurements and serum albumin concentrations, those which Bistrian et al used in their nutritional survey of surgical patients (2), are accepted worldwide (5). Using these parameters a number of patients were considered to have become acutely malnourished during their stay in hospital. This, it was claimed, was due to a combination of their catabolic condition (injury, surgery of infection) and infusions of 5% dextrose as the sole element of nourishment (3). Serum concentrations of albumin and transferrin were found to have dropped markedly. Cell mediated immunity showed impairment and there was a depression of total lymphocyte counts in peripheral blood.

Hill and his colleagues have also commented on the failure to recognise protein-calorie malnutrition in patients in general surgical wards, particularly in those assessed more than a week after major surgery (4). Of their patients less than 5% had been given any sort of nutritional therapy, confirming the view that conventional intravenous therapy after major surgery, with its

emphasis on water and electrolyte balance, contributes very little to the nutritional needs of surgical patients. A tacit gesture towards nutrition is made by giving low calorie glucose solutions.

Certainly the lack of suitable alternative solutions until recent years, the failure to appreciate the importance of continuing adequate protein intake and the mistaken assumption that what has been demonstrated in normal human volunteers holds true for the stressed post-surgical patient have all played their part in the current practice of limiting the range of fluids given to patients unable to eat or drink after surgery to combinations of 5% dextrose and various electrolyte solutions. Gamble carried out a series of tests on normal volunteers and demonstrated that small amounts of carbohydrate were nitrogen sparing (34), but this amount, 50 - 100 grams of glucose, infused during period of sepsis or after major injury takes no account of the insulin resistance current in that situation. Even Randall in a review paper on fluid, electrolyte and acid-base balance, fails to draw the distinction between the nitrogen sparing effect of carbohydrate in starvation and after moderately stressful surgery (35). Many days may pass before post-operative recovery can be compared to unstressed semi-starvation.

It is relevant before discussing some of the effects of surgery on man to comment on studies in undernourished children fed on diets containing little or no protein. In such marasmic children, already depleted in their reserves, but with a relatively normal proportional body composition, the feeding of carbohydrate leads to development of kwashiorkor. There is expansion of the extra-

cellular fluid compartment, lowered plasma albumin concentration and development of pitting oedema and ascites (36). A similar natural progression is observed in the development of the "sugar baby' syndrome, an appropriate name for kwashiorkor in West Indian children, who, having been weaned, are left to subsist on inadequate protein diets, but are permitted to chew sugar cane (36). Chronic or recurrent infection may also tip children who exist on a barely adequate diet into a kwashiorkor state (37).

If such diets in children cause kwashiorkor, it is a reasonable assumption that the effect of glucose alone in adult patients in hospital will tend to produce changes along similar lines. The rate at which a kwashiorkor-like syndrome will develop will depend on a number of variables such as the initial nutritional status, the presence of infection, the surgical or other catabolic stress and the period of nutritional deprivation. Some patients have already become marasmic from their illness or from failure to eat or absorb food because of it. Such patients are likely to become severely nutritionally depleted more rapidly than patients with normal reserves.

Elwyn wrote of his concern in 1973 (33) and subsequently has carried out whole body water studies in which he has demonstrated abnormalities in the distribution of water with patterns characteristic of kwashiorkor. He has proposed that the changes were due to the administration of 5% dextrose (38).

The total exchangeable potassium in the body is a measure of the body cell mass which has been defined by Moore as "that component

of body composition containing the oxygen-exchanging, potassium rich, glucose oxidising, work performing tissue" (39). A method of measuring the total exchangeable potassium is described by Shizgal and with it he showed that in catabolic surgical patients the body cell mass is markedly contracted and that the extracellular component is expanded when compared to normal volunteers. Similar changes, though less in extent, were recorded after major abdominal surgery in patients whose intravenous fluid regimen contained only glucose (40).

Thus by well accepted criteria of nutritional assessment, by whole body water isotope studies and by other isotope studies to measure body composition relating to the body cell mass, it is concluded that the administration of 5% dextrose to patients after surgery leads to changes in water distribution which tend towards the kwashiorkor end of the malnutrition spectrum. Nevertheless the experience of most surgeons is that patients recover well from uncomplicated surgery, even if moderately severe, and return to normal nutrition after a reasonably short interval. This is particularly so if they appeared to be normally nourished beforehand. The important point to learn from the above studies is that if there is a prolonged delay in the restoration of normal nutrition after surgery, there will be an inevitable progression towards a protein deficient state. Whether this is recognised clinically, or indeed will be of clinical significance, will depend on the vigilance of the staff, and the many other factors mentioned previously. Its reversal can be achieved by attention to nutritional therapy. Often it is not possible or advisable to use the oral or enteral route. In these patients one of the

techniques of parenteral nutrition can be initiated to lessen or overcome the continuing protein catabolism and loss of nitrogen. These techniques of intravenous hyperalimentation (IVH) and protein sparing therapy are discussed below.

INTRAVENOUS HYPERALIMENTATION

The development of the practical approach for intravenous hyperalimentation by Dudrick (41) provided a method of restoring or maintaining the nutritional status of patients in hospital. By this technique hypertonic dextrose and protein solutions (when first used these were protein hydrolysates, but currently synthetic crystalline amino acid solutions are available) are infused into a central vein together with electrolytes, vitamins, trace metals and other essential minerals (42). There are now many varied amino acid solutions available commercially with minor or major differences in composition. Some of these are combined with a carbohydrate source of energy. Fat emulsions and non-glucose carbohydrates such as sorbitol, ethanol or fructose can also be obtained. Many of these latter have disadvantages, the risk of lactic acidosis being but one. The use of intravenous hyperalimentation is now reasonably well established in the obviously malnourished and its development has led to well written textbooks being published advising on indications, techniques, complications and modifications in such clinical conditions as hepatic or renal failure (43, 44). International conferences and workshops take place to discuss the latest advances (45, 46).

There is however much discussion about the most satisfactory composition of solutions, regimens to be adopted and the effect

of varying the proportions of nitrogen infused to energy available for anabolism. Two years after the clinical work for this thesis was finished, Young and Hill urged the need for further investigations to assess the value of intravenous hyperalimentation in relation to the accepted nutritional parameters (47). The first study in this thesis goes part of the way to meet this need as the relative efficacy of IVH and enteral nutrition were compared in malnourished patients using weight change, nitrogen balance and changes in serum albumin and transferring concentrations as the criteria. The relevance of transferrin was of especial interest as it was postulated that an improvement should be evidence in a period of two weeks due to the more rapid turnover and distribution whereas this was not expected for albumin due to its longer half-life and greater proportion found in the extravascular compartment.

Intravenous hyperalimentation is not a technique to be used without careful thought. The possibilities of complications from catheter insertion or infection (48, 49) and the incidence of venous thromboses (49), even though these complications are very much less frequent in the hands of trained hyperalimentation teams (48), make it necessary for a clear indication for intravenous hyperalimentation to exist before it is used in any specified patient. Certainly it will not be needed by every patient who is unable to eat for a few days. Indeed depending on the nutritional status of the patient there may be no need for IVH even in one who cannot return to normal nutrition for two or three weeks. Perhaps the ultimate factor which will limit the widespread use of intravenous hyperalimentation will be the prohibitive cost.

PROTEIN SPARING THERAPY

Another nutritional technique which has been developed over the last decade is that of "protein sparing therapy" which is based on the recognition that in starving or fasting man certain metabolic adaptations occur by which there is a shift from the use of carbohydrate as the main energy source to fat. The metabolites of free fatty acid breakdown in the liver, the ketone bodies acetoacetate and β-hydroxybutyrate are used by tissues to supply energy (50). Protein sparing therapy has been proposed by Flatt and Blackburn (51) and is as applicable to the oral route in the reduction of obesity, particularly in diabetic adults (52, 53), as it is by a parenteral route in the post-operative management of surgical patients. The critical feature is the administration of "protein" with no carbohydrate as will be discussed below.

It is particularly with surgical patients that this thesis is concerned. "Protein" is supplied by crystalline amino acid solutions which can be infused in isotonic concentrations into peripheral veins. As centrally placed venous cannulas are not necessary, many of their potential complications are avoided. However these solutions are hypocaloric and do not supply the optimal energy requirements for anabolism which are provided by intravenous hyperalimentation. Nevertheless very acceptable nitrogen balances can be achieved, particularly in the relatively malnourished (54).

The physiology of the changes occurring in fasting is first discussed. In the fasted state the hepatic stores of glycogen

are exhausted within 24 hours. There is no other carbohydrate reserve. Glucose, which is necessary for oxidation, is synthesised from glucogenic amino acids in the liver. The source of these is muscle protein which is broken down and the amino acids are released into the circulation. Another mechanism also exists through which glucose production continues, the Cori cycle. This is the process by which lactate and pyruvate produced in many tissues by the metabolism of glucose particuarly in skeletal muscle, heart and renal cortex are recycled to the liver where they are resynthesised into glucose using energy derived from fatty acid oxidation. If there was no Cori cycle activity there would be greater muscle breakdown to provide glucogenic amino acids, hence the cycle can be considered to be protein sparing (50).

With a falling glucose concentration in the plasma, the stimulus for insulin release from the pancreas is reduced and hence the inhibitory action of insulin on fatty acid mobilisation from adipose tissue is lowered. Free fatty acids released from the fat stores can be used directly by some tissues or are oxidised to ketone bodies in the liver. The latter can be used by many tissues and may meet up to 70% of the energy requirements of the brain (55). Full adaptation to the fasting state may take some three weeks, by which time substantial muscle breakdown will have occurred (50).

In a brief period of fasting Cahill considers that insulin plays a leading if not fundamental role in regulating the release of body fuels from the peripheral tissues (56). One of its actions

is to lower the concentrations of cyclic AMP in adipose tissue and hence decrease the activity of the lipolytic enzyme responsible for initiating triglyceride hydrolysis (57). Insulin has many other effects on responsive cells, altering membrane characteristics to allow the transport of glucose, amino acids, potassium and other ions. It alters ribonucleic acid and protein synthesis within cells and may have an inhibitory effect on proteolysis in muscle (56). Insulin release is mediated through the beta cells of the pancreas which are very sensitive to small changes in glucose concentration and can produce a rapid response to those changes. A negative feedback control over fatty acid mobilisation exists as both fatty acids in high concentration and ketone bodies appear to stimulate insulin secretion directly (58). In this way lipolysis is controlled and ketoacidosis is prevented.

Other hormones have roles to play in fasting. The adrenal glucocorticoids and growth hormone exert an anti-insulin effect and may facilitate fatty acid mobilisation (50). Glucagon has a much more major role. In man, fasted for two to three days, rises in circulating concentrations of glucagon were reported by Aguilar-Parada et al (59). Glucagon, secreted by the alpha cells of the pancreas, has an action antagonistic to insulin in promoting glycogenolysis and gluconeogenesis, in the liver, and in mobilising fatty acids from adipose tissue. These are essentially adaptations to the "stress" situation where body fuels are rapidly required. When small doses of glucagon are administered to fasting man, a decrease in the concentration of amino acids occurs, perhaps by augmenting peripheral uptake, yet without changes in

insulin or glucose concentrations (59). This suggests that glucagon has a very fundamental role in maintaining the balance of circulating amino acids and hence an important role to play in the supply of body fuels during starvation.

Ungar has suggested that it may not be the absolute concentration of glucagon which is of importance, but rather the insulin/glucagon ratio (60). Whatever the metabolic situation, whether in fasting or after trauma or infection the insulin/glucagon ratio will be such as to "mediate the disposal of amino acids as efficiently as possible". The insulin/glucagon ratio varies inversely with the need for glucose production and hence in total starvation is at its lowest.

THE METABOLIC FUEL REGULATORY SYSTEM

Recognition of the role which ketone bodies play as an energy source both in starvation and semi-starvation led Flatt and Blackburn to propose their metabolic fuel regulatory system in which the inter-relationships of the various body fuels, glucose, amino acids, free fatty acids and keton bodies, and their concept of insulin as the key controlling hormone were emphasised (51). Glucose concentrations are elevated following either administration or secondary to endogenous production from amino acids by their conversion into glucose in the liver and to a lesser extent in the kidney (61). The stimulus for insulin secretion is thus directly or indirectly mediated. However certain of the amino acids exert a direct stimulatory effect on insulin release, but this is more effective when glucose concentrations are relatively elevated (62).

According to the regulatory system glucose, more than the other metabolic fuels, is held to influence insulin levels. This view is perhaps crucial to the development of protein sparing therapies. In starvation, with the adaptation already discussed, there are low glucose levels. Once a small amount of food is eaten, there exists a state of partial starvation. Carbohydrate, which raises glucose concentrations, stimulates insulin release. Amino acids alone have a reduced insulin stimulatory effect partly because of the indirect conversion to glucose but also as direct stimulation is attenuated in the low prevailing glucose concentration (63). Thus when compared with an equal amount of carbohydrate ingestion of protein alone will provoke a lesser insulin response and hence will cause less inhibition of fat mobilisation.

Gamble showed that giving 50 - 100 grams of carbohydrate per day will reduce ketosis and will spare protein breakdown (34). This amount which corresponds to the infusion of one to two litres of 5% dextrose solution per day supplies glucose for oxidation which would otherwise have to come from proteolysis. However the rise in insulin reduces fatty acid release from adipose tissue, and hence slows down the formation of ketone bodies with consequent reduction in the energy thus made available. It can be argued that giving this small amount of carbohydrate over a period of some weeks has a deleterious effect because the adaptation to the use of fat is prevented. If energy which must be supplied is unavailable from fat metabolism, it will come from protein metabolism, which effectively means muscle catabolism.

As the stimulus for insulin release is reduced with protein or amino acid feeding, ketone body production should be increased. Blackburn and his colleagues have substantiated this proposition by their demonstration of similar metabolic profiles in fasting and in patients given 0.6 - 1 gram protein per kilogram body weight per day (64). Further, they found that the amount of urinary nitrogen excreted was comparable and concluded that the rates of gluconeogenesis were similar whether with or without the limited intake of protein. Thus the administration of this small amount of protein did not interfere with the metabolic adaptation to fasting with its presumed evolved significance of preserving the body cell mass. By supplying amino acids, there would be restoration of those proteins degraded and a nitrogen balance which would approach zero. This expectation was also confirmed (64).

It is the extension of this work by the use of amino acid solutions infused intravenously which has led to much interest in surgical practice. Others than Blackburn and his colleagues (65, 66, 67) have confirmed that with amino acid infusions, free fatty acid and ketone body concentrations rise to levels which approximate to those found in fasting individuals (68). Most of these corroborating studies have been carried out in the period after surgery to which the greatest application in surgery of protein sparing therapies would seem to apply. However surgical trauma, as any stress, has profound and often long lasting effects on metabolic processes. These will now be discussed.

THE METABOLIC RESPONSE TO STRESS

Virtually any insult to an individual whether it be physical, traumatic, surgical or infective will provoke a stress response from that indivudual. Although first described by Sir David Cuthbertson in 1930 (69) in patients with bony and soft tissue injuries, it is now accepted that the metabolic response is essentially the same whatever the insult. Traditionally the response has been considered in terms of fluid and electrolyte balance, but in the past few years the nutritional aspects have been emphasised. It is not the purpose of this brief commentary to discuss extensively the various changes which occur or their controlling mechanisms. These have been reviewed elsewhere (70, 71, 72, 73, 74). Rather it is proposed to summarise the changes with respect to nutritional needs and to discuss a few of the metabolic changes in order that the concept of protein sparing therapy as described above, and the work to be reported later, are put into context.

The initial response, variously described as the "ebb" phase (70) or acute phase (75), either in anticipation of the insult or induced by it, is a central nervous system mediated sympathetic discharge with release of catecholamines from the adrenal medulla. The anticipatory reaction to "fight or flight" was described by Cannon in 1929 (76). The neuroendocrine response is mediated through many hormones, the catecholamines as mentioned above, growth hormone, antidiuretic hormone, adrenocorticotrophic hormone, cortisol, aldosterone, thyroid stimulating hormone, glucagon, insulin and the androgens (74). The extent and duration of the response is related to the severity of the injury with, in

general, an increased release of all the above hormones except for insulin and the androgens.

The nutritional effect of this response is to mobilise the body fuel stores. Liver glycogen is converted to glucose through direct sympathetic innervation of the liver and secondary to the action of circulating adrenaline and glucagon, the latter being released through sympathetic stimulation of the pancreas. Muscle glycogen is also converted to glucose by the action of adrenaline and non-adrenaline. Glucagon not only inhibits hepatic glycogen synthesis and stimulates glycogenolysis but also promotes the uptake of gluconeogenic amino acids into the liver and their conversion to glucose. Glucose synthesis from lactate, pyruvate and glycerol is also stimulated. Glucagon has no demonstrable effect on skeletal muscle protein metabolism (72) but free fatty acid release from adipose tissue is increased by both glucagon and the catecholamines.

Growth normone may also increase lipolysis and reduce glucose utilisation by peripheral tissues. The glucocorticoids promote increased amino acid release with a corresponding decrease in uptake of amino acids and protein synthesis in muscle and many tissues. They also stimulate hepatic enzyme synthesis. In this way the circulating amino acid pool is replenished (77), providing substrate for oxidation and also making amino acids available for the many processes such as blood protein synthesis, tissue repair, maintenance of immune function and acute phase protein synthesis, all of which will require a substantial supply of substrate. There is an outpouring of nitrogen, largely as urea from protein

catabolism, and the individual goes into a negative nitrogen balance.

In the initial ebb phase there is a reduction of metabolic activity despite the apparent abundance of energy producing substrates in the blood. Hyperglycaemia is persistant due to diminished glucose utilisation by tissues (78). This early effect, which has been demonstrated during surgical procedures (79), is probably due to two complementary mechanisms. The increased sympathetic activity inhibits the secretion of insulin and hence there is a relatively low insulin for the high circulating glucose concentration (72). There is also insulin resistance due to some of the hormones, although how this mediated on a cellular basis is not understood. Both growth hormone and the glucocorticoids are antagonistic to the action of insulin. Thus tissues which normally would take up glucose under the influence of insulin, are less sensitive under the conditions of insulin resistance. Johnston has shown that the plasma concentrations of cortisol rise during surgery and remain at a peak for some three to four hours, then fall off (80). However the concentrations of the metabolites remain elevated for much longer than cortisol which suggests that the interplay between conjugation, metabolism and excretion may be relevant to the activity of cortisol and its induced insulin resistance.

One can but speculate on the significance of these observations. From an evolutionary point of view this may be a mechanism by which an individual mobilises, but preserves its body fuels as its ability to obtain suitable food may be limited for an uncertain period.

A further feature of the "ebb" phase is that of decreased heat production. The mechanism is not understood but may be due to disturbance in the thermal regulatory centre (73). Hormonal changes may also play a part as indeed impaired glucose utilisation. This latter may be overcome in the clinical setting by the judicious use of glucose with supplemental insulin.

Very early changes in the concentration of thyroid stimulating hormone occurs after stress (80). The contribution which the thyroid hormones make in energy production in the "ebb" phase is uncertain if it is the case, as put by Johnston, that rapid secretion of thyroid stimulating hormone is closely followed by a rise in the concentrations of thyroxine and tri-iodothyronine (80). If so, one would assume that there should be a rise in the temperature as these hormones increase the metabolic rate and oxygen consumption. However on closer scrutiny of the method of assaying the hormones it would seem that there is an increase in the free thyroxine index with reduced thyroid binding proteins. This latter point as it relates to prealbumin will be commented on later. In short, the precise mechanisms by which heat production in the "ebb phase" is depressed are complex and not clearly defined.

The transition from "ebb" to "flow" phase takes place over a variable length of time depending on the severity of the insult, but normally in uncomplicated situations this has occurred by one to three days after the injury. The "flow" phase is characterised by increased heat production, increased basal oxygen consumption, increased protein catabolism, continuing insulin resistance

and ketosis. It is during this phase that recovery and repair start and that the largely catabolic processes gradually tail off and net anabolism supervenes. The response to injury may be modified by the environmental temperature. Cuthbertson has shown that the response which is greater in the more severely injured was less at a higher temperature (81). In a further study he showed that patients nursed in a higher ambient temperature had reduced protein catabolism (82).

During the "flow" phase there is a decline in the concentration of growth hormone and of the glucocorticoids. Insulin resistance lessens and tissue sensitivity thereby increases. Glucose is more readily taken up, lowering the plasma concentration. The falling glucose concentration is taken as one of the useful indices of the arrival of what Blackburn calls the adaptive phase (76), which is not synonymous with Cuthbertson's "flow" phase, but with whose advent it is possible to initiate protein-sparing therapy or other nutritional techniques.

The duration of the "flow" phase is also variable. Return to normality is fairly rapid after uncomplicated surgical trauma. However if trauma has been severe or if sepsis exists, the "flow" phase may be prolonged with continuing loss of nitrogen, which may be lethal if it continues to the extent that 30 - 50% of body protein is lost. The most marked catabolism occurs in patients with very severe burns who may approach this limit within a few weeks, with a loss of up to 25 grams of nitrogen a day (72). This loss is equivalent to 156 grams of protein or 0.625 Kg of lean muscle.

As yet it has been assumed that the loss of protein has been from skeletal muscle. In the body there is some 4.5 kilograms (assuming a body weight of 70 kilograms). Non-carcass or "visceral" protein amounts to a further 1.5 kilograms and this together with the plasma proteins could also be the source of amino acids. The effect of stress on the plasma proteins will be discussed in a subsequent passage. The suggestion that muscle was broken down was made initially by Sir David Cuthbertson with his observations that the increased urinary excretion of phosphate, sulphur and potassium after injury approximated to the proportions found in lean muscle (69). Further studies which he carried out with zinc excretion after trauma have also supported this view (zinc being normally found in muscle) (81). In his review of the metabolic adaptation after trauma and sepsis Ryan discusses some of the more recent studies (72), mostly on rats, in which the source of the amino acids is confirmed as muscle. It is of interest that in the rat, the effect of trauma and sepsis with the relative preservation of the liver mass is contrasted with the effect of fasting in which visceral protein (of which liver protein contributes the greatest proportion) is depleted before muscle protein is utilised.

That increased catabolism occurs after trauma is not seriously disputed, although O'Keefe and his colleagues did argue that the loss of protein was due to decreased synthesis, hence failure to recycle amino acids with their subsequent breakdown (83). They used ^{14}C labelled leucine to measure protein synthetic and breakdown rates and concluded that there was a reduction in

protein synthesis without an increased breakdown. Two points can be made about their conclusions. The first is that as the study was carried out only two days after surgery, it cannot be assumed that the patients had moved fully into the "flow" phase and in the "ebb" phase there is no dispute that protein synthesis is depressed. The second is that they were unable by their technique to define to which protein compartment their observations were relevant as they measured net rates of synthesis and breakdown.

Metabolic expenditure in uncomplicated patients may not alter over a period of moderately severe surgery. This has been shown by Tweedle and Johnston (84) and commented on by Clark (85). Although the total expenditure does not alter, the resting metabolic expenditure does increase, but the active expenditure of energy is reduced. The action of thyroid hormones is probably one of the related factors. Oppenheimer's group have not confirmed Johnston's observation that there is a rise in thyroxine and tri-iodothyronine after surgery. They noted a decrease in the active hormone, tri-iodothyronine, but not thyroxine after elective surgery (86, 87). The free indices of the hormones were however raised. Similar observations were also made in other diseases in which there was an inflammatory or infective element. As the free thyroxine and free tri-iodothyronine indices were raised it is easier to understand how they could make a contribution to heat production which is known to be increased in the "flow" phase.

Traditionally the action of the thyroid hormones has been considered

to be mediated in the mitochondrion. However many of the in vitro experiments have been done with concentrations some thousand times that found in vivo, which requires them to be put into perspective (88). Oppenheimer and his colleagues have produced very convincing evidence that there are also binding sites in the nuclei of cells, and it is at these sites that the thyroid hormones, particularly tri-iodothyronine, act (89). Two major roles are proposed for tri-idiothyronine (86). The first is the stimulation of membrane bound sodium/potassium ATPase activity with a net consumption of oxygen and heat production. The close relationship between the energy required for maintenance of the intracellular and extracellular gradients of sodium and potassium and the heat produced adds support to this role. The second role is of DNA transcription in the nucleus which is facilitated by tri-iodothyronine. By this means new protein synthesis primarily of enzymes is initiated. In one study using two hepatic enzymes the increased enzyme activity was correlated with an increased enzyme mass (90).

Thyroxine itself would seem to have little biological activity. Some 90% of the binding sites are occupied by tri-iodothyronine (86). In catabolic illness however, when tri-iodothyronine concentrations are depressed, the clinical state of hypothyroidism is not found. This suggests that thyroxine may have some intrinsic activity which is not as yet understood, as the thyroxine concentrations do not decline.

Increased metabolic expenditure in the "flow" phase with increased heat production may be seen to be related not only to the increased

glucose utilisation which follows the reduction of insulin resistance, but also may be due to the action of thyroid hormones which, even if reduced in their serum circulating concentrations, are less bound and may act more efficiently at the cellular level, producing heat and increasing protein synthesis in tissues such as the liver which are sensitive to the action of these hormones.

From the nutritional point of view the "flow" phase can be considered the period in which mechanisms exist to preserve nitrogen, in that fat becomes the main source of energy with the gradual cessation of muscle breakdown. It is a transitional phase, in which anabolism is initiated with a ready supply of amino acids made available together with glucose for production of energy once insulin resistance lessens. Indeed once nutrition is established, repair and recovery proceed.

It is worth highlighting certain aspects of the metabolic response to stress as they relate to the common practice of infusing 5% dextrose, which, it is suggested, can be an unnecessary and potentially harmful practice. So few calories are supplied by glucose, particularly when the extent of the mobilisation of the body fuels is recognised, that the contribution from the solution infused is negligible. The extent of insulin resistance anyhow makes it unlikely that such glucose is efficiently utilised. Even after the resolution of insulin resistance, the effect of infusing glucose is to stimulate insulin secretion which alters the balance of fuel mobilisation away from fatty acid and ketone body production and hence from that adaptation which the body would

seem to have evolved to spare its protein from breakdown.

Some of the glucose infused will require to compensate for reduced ketone body oxidation, and as such can be considered to "spare" fat. Further by the action of insulin in enhancing amino acid uptake in skeletal muscle, particularly once tissue resistance to insulin has declined, there is less amino acid substrate available for protein synthesis in the liver and other visceral organs. Thus by enhancing skeletal muscle uptake other synthetic pathways may be compromised. It may therefore be argued that the metabolic response to injury has evolved to ensure that there is a ready supply of amino acids for the "visceral" synthetic processes which are stimulated by injury and which are essential for survival, and that this supply has some degree of priority, even at the expense of skeletal protein. It is however not claimed that the amino acids made available are solely used for visceral synthesis, rather that these processes which are essential do not fail from lack of the basic materials but can be switched on or continue at a much more rapid rate.

THE EFFECT OF STRESS ON PLASMA PROTEINS

As with the metabolic response to stress, there is a non-specific response of the plasma proteins to any insult to the body whether severe trauma, surgical intervention or even myocardial infarction. A number of the proteins which show early increases in concentration have been termed the acute phase proteins. Those are mostly found in the α_1 and α_2 globulin fractions and their pattern of rise and fall is predictable, unlike other proteins such as albumin and transferrin whose concentrations decrease

(8, 15, 91, 92, 93, 94). Fibrinogen is a further protein to show rises in concentration but no further mention will be made of it as it is the effect of stress on possible "nutritional marker" proteins secreted by the liver with which this thesis is concerned whereas the metabolism and turnover of fibrinogen is complex. Although it is known that there is increased synthesis of RNA followed by aggregation of polysomes in the liver following injury, thus increasing the secretory ability of the liver, the mechanisms by which the proteins are produced, the priorities for synthesis or release and sites of degradation remain largely undefined (15). Local hormonal concentrations, nutritional status and supply, and factors released by damaged tissues are all likely to play a part. It has been suggested by Koj and McFarlane (95) that the common event in stress is the disruption of lysosomes with the enzymes released either stimulating synthetic pathways or, more likely, lysing specific inhibitors to permit more rapid synthesis to occur.

Powanda (96) has recently suggested that the host response to inflammatory stress should be understood in terms of redistribution of nitrogen from peripheral to visceral tissues for various aspects of host defence, in particular for the synthesis of acute phase proteins. The proposed trigger for this redistribution is phagocytosis with the consequent release of factors such as pyrogen and leukocytic endogenous mediator. By this means, it is suggested, the body has developed an adaptive mechanism to protect organs with vital functions in the face of the catabolic effect of stress and infection.

Before considering the acute phase proteins in greater detail, a review is pertinent of the effect of stress on the specific nutritional "marker" proteins. The drop in albumin concentrations has already been mentioned with the likely explanation of redistribution into tissue spaces with increased degradation and the postulate that it might act as a feeder protein (8, 16). Fleck quotes a figure for the ratio of extra-vascular to intra-vascular albumin changing from the usual of around 1.5:1 to 2:1 (73). He also comments on the failure of several investigators to detect an increase in the absolute catabolic rate of albumin after minor or moderate injury, although there was an increase in the fractional catabolic rate, that is the fraction of the intra-vascular pool catabolised.

Skillman and his colleagues (97) made the interesting observation that the synthetic rate of albumin remains normal or is enhanced if appropriate nutrition is given after surgery. A criticism of their study could be that they did not measure synthetic rates before surgery on their patients. Despite the improved synthetic rate, the concentration of albumin fell after surgery in groups given either amino acids or glucose, a feature which is in accord with the above observations on redistribution. There are many influences on the synthesis of albumin after injury including haemorrhage, hormonal balance and nitrogen intake (6, 7) and clearly the plasma concentration after injury reflects some small part of these diverse factors. In this respect Shillman's results contrast with those of O'Keefe (83) who claimed there was decreased protein synthesis after surgery, although it is acknowledged that Skillman's work was specific and O'Keefe's general.

Transferrin concentrations also decline after "stressful" events, dropping to 78% of pre-operative values eight hours or more after minor surgery (15). Whether there is a greater diffusibility allowing a more ready escape from the plasma to the sites of degradation as considered by McCathie et al (91) or turnover is increased, is unknown. It is reasonable to assume that, as the synthetic mechanism for transferrin appears to be similar to that of albumin, there is similarly no decrease in its synthetic rate.

The response of transferrin to cholecystectomy, as also that of 18 other proteins was measured by Aronsen et al (93). Using pooled serum from 100 healthy blood donors as a standard, they showed a decrease to a mean of 69 - 80% of this on the third to fifth post-operative days with a gradual return to pre-operative concentrations by the end of three weeks. In comparison the percentage fall of albumin was less, but the return was as prolonged.

Prealbumin concentrations were also studied. The nadir was on the third post-operative day at a mean level of 52% of the pooled serum concentrations with which the pre-operative levels closely agreed. Return to normal was almost complete by two weeks. A comparable fall after mastectomy was also observed. These results were obtained after fairly uncomplicated surgery on patients in whom it is considered there was a fairly rapid return to normal nutrition. As such the changes in both prealbumin and transferrin concentrations could be considered as "stress induced" rather than due to nutritional depletion.

Surgery is not the only stressful event to cause a drop in prealbumin concentrations. While investigating the binding of thyroxine in non-thyroid illness, Oppenheimer and his colleagues noted that in patients with such diverse conditions as ulcerative colitis, myocardial infarction and abscess formation there was a marked fall in the maximal binding capacity of prealbumin for thyroxine, but concluded that this was secondary to a drop in concentration of the protein rather than a reduced affinity of prealbumin for thyroxine (98).

To account for the greater fall of prealbumin (M W 60,000) than albumin (M W 69,000) with that of transferrin (M W 90,000) intermediate between the two, some mechanism in addition to that of redistribution between the intra and extra-vascular spaces should be postulated as relative falls in concentration would be expected to be inversely proportional to the molecular weight of the protein. There may be selective transport, but this is unlikely because of the size of the molecules. The formation of a complex has been suggested. For transferrin there may be some relationship to the early increased uptake of iron by reticular cells which is part of the inflammatory response. However no experimental evidence has been put forward demonstrating that transferrin or a complex with transferrin is taken up by these cells (93). For prealbumin there may be a carrier function by which a complex is held in tissues rather than maintaining the "non-stressed" equilibrium between the various compartments. This is unlikely to be related to thyroid hormone function because of the minor role in thyroxine binding, but could be related to vitamin A metabolism after injury. However the drop

in prealbumin concentration would be expected to be greater than for the other proteins because of its smaller molecular weight, and hence greater likelihood of diffusion through permeable membranes, and as such there is no need to invoke a separate transport function.

THE ACUTE PHASE PROTEINS

Reference has already been made to this group of proteins who derive their name from the fact that their "serum concentration is increased in the acute phase of inflammatory states" (8). They are all glycoproteins with from 5 - 20% carbohydrate in their structure and all are synthesised in the parenchymal cells of the liver. Although the precise mechanisms by which infection or injury stimulate increased synthesis and secretion, the priority which such synthesis has over other metabolic pathways is demonstrated by the finding of an appropriate acute phase protein response in malnourished children (99), and in starved and infected animal models (100, 101). As the supply of dietary protein has been shown to be a factor in the synthesis of other proteins secreted by the liver, the synthesis of the acute phase proteins is also likely to be dependent on amino acid availability (102), possibly using similar pathways. Because of the overriding priority of this response in inflammatory stress, mechanisms exist to ensure an adequate prompt supply of amino acids into the hepatic parenchymal cells. Comment has already been made under "The Metabolic Response to Stress" indicating that such a flow of necessary substrate exists.

Powanda's proposal that the acute phase protein reaction is

triggered by factors derived from granulocytes or fixed cells of the reticulo-endothelial system involved in phagocytosis has experimental support (96). Leucocyte derived factors increase the movement of amino acids out of muscle and enhance the flux of amino acids into the liver (103), findings which are consistent with clinical observations that muscle is catabolised during infection.

The acute phase proteins which were assayed in the work to be described later are introduced briefly in their functional capacity in which they could be considered to play their part in the redistribution of nitrogen for host defence.

Orosomucoid (α_1-acid glycoprotein) appears to interact with platelets to aid their binding to collagen and direct the formation of collagen fibres (104), thus playing a part in wound healing. α_1-antitrypsin, by its inhibition of trypsin, chymotrypsin and other vasoactive peptides (105), may limit the damage to tissues surrounding the site of reaction between leucocytes and micro-organisms.

Haptoglobin, an α_2 glycoprotein, has the capacity to complex with haemoglobin. This complex is removed by reticuloendothelial cells from the site of haemolysis, thus conserving iron and preventing iron deposition in renal glomeruli (106). It has been suggested that 50% of the haptoglobin synthesised is drained from the circulation because it complexes with haemoglobin (93).

Caeruloplasmin is the major protein to which copper is bound (107),

but it has also been shown to be an enzyme capable of oxidising catecholamines and serotonin and of binding histamine. It may thus be the means by which histamine may enhance the catabolism of potent vasoconstrictors. Another of the acute phase proteins, α_2 macroglobulin, is the principal carrier of zinc in human serum (108). It also inhibits protease (109). Its roles may be to modulate various aspects of the host response to infection and assist in wound healing. However, its concentration does not increase after injury or infection (92, 93, 94). The function of C-reactive protein, which is the earliest of the acute phase proteins to rise, has been proposed to be a promoter of phagocytosis (110).

Thus the acute phase globulins maintain the balance between levels of vasoconstrictors (caeruloplasmin), prevent renal damage by scavenging iron (haptoglobin), aid wound healing (α_1-acid glycoprotein), minimise vasodilation, clotting and tissue damage (α_1-antitrypsin and α_2-macroglobulin) and promote phagocytosis (C-reactive protein). The probability exists that there are many more functions of these proteins and that numerous controlling mechanisms with feedback, both positive and negative, can vary the response of the system to the differing stressful events. Few, if any, studies have been carried out to examine the nutritional effect of administering amino acids to animals or humans exposed to infection, surgery or other stress in order to monitor the pattern of response of the acute phase proteins. One of the purposes of the second study in this thesis is to carry this out by determining whether supplying amino acids modifies the acute phase protein response to surgery.

Constant replenishment of the amino acid pool has the theoretical benefit of providing necessary substrate for protein synthesis. However as the acute phase response appears to have an over-riding priority in the synthesis of new protein in inflammatory stress, in which considerable quantities of amino acids are released from muscle, the addition of amino acids by infusion may not influence any of the pathways. Ideally, synthetic rates of these proteins would be measured prior to a stressful event, and during infusions with and without amino acids. This is currently an impossible technical task, and conclusions, albeit crude, will have to be drawn from assays of plasma concentrations.

RATIONALE FOR AMINO ACID SOLUTION

The most tested and most accepted method of assessing any nutritional technique is nitrogen balance. Isotonic amino acid solutions infused in the post-operative period have been shown by many groups to produce much improved nitrogen balance when compared with that during dextrose infusions of similar calorie content (64, 65, 66, 97, 111, 112, 113). There is however less agreement about any benefit conferred by supplementing amino acid solutions with either carbohydrate or fat emulsions, or about the mechanisms by which protein is spared. For example, Greenberg, et al. were unable to show any difference between the nitrogen balances obtained solely with amino acids and when they were infused together with 5% dextrose or with Intralipid, a fat emulsion (65), although all these solutions showed marked improvement over glucose alone. Their findings appeared to conflict with those of Blackburn's group whose patients on glucose/amino acid infusions did not match the nitrogen balance of those solely on amino acids

(112) and they therefore challenged the metabolic basis on which the concept of protein sparing therapy had been developed. Flatt, in defence of the metabolic fuel regulatory cycle, deplored the failure of Greenberg's group to take account of its "flexibility in accommodating variations in metabolism and response to nutritional intake encountered after trauma or during disease" and pointed out some of the inconsistencies in their argument (114).

Freeman and his colleagues too were unable to show any differences in the nitrogen balance by the infusion of amino acids or in combination with 5% dextrose (115). They noted metabolic fuel profiles (free fatty acid, ketone body and glucose and insulin concentrations) in the latter group which had reverted to those while the patients were on glucose alone and suggested that the importance of a low circulating insulin which had been emphasised by Blackburn's group was over-emphasised. However far from concluding that their results proved that lipolysis was completely inhibited by glucose, they commented that the reduced lipid metabolites merely reflected that an alternative energy source was being provided and less fat required to be broken down for the energy needs. In essence they were illustrating the point made by Flatt (114).

As no part of the study to be described later involves the infusion of combinations of isotonic amino acid and dextrose solutions as part of a study of protein-sparing therapy, it is not proposed to continue the above discussion apart from making two points. The first is that there is only limited value in initiating any nutritional therapy in the immediate post-operative period. This

aspect is often ignored in studies of protein sparing after surgery. It is only once the initial high glucose concentration has declined and ketosis has developed that a state of semi-starvation analagous to fasting is obtained and amino acids can be most effectively utilised. As most of the studies reported cover the first three to four days after surgery when the stress is at its greatest and most variable, and the metabolic environment is least stable, it is hardly surprising that different results are obtained and from them differing conclusions drawn. Unfortunately this criticism is valid for one of the studies to be described later, but of necessity is a feature when projects are undertaken in patients undergoing surgery in units where there is a great demand on available resources.

A second more practical point should also be made. In no study has improved nitrogen balance ever been demonstrated by the supplementation of amino acids with 5% dextrose or fat emulsions. The addition of such a dextrose solution to isotonic (3 or 3.5%) amino acid solutions virtually doubles the osmolality, to above 600 mOsm/l. Peripheral veins are unlikely to tolerate the infusion of these hypertonic solutions for long, and as one of the benefits of protein sparing therapy is that the solutions are given peripherally, thus obviating the risks of central venous catheterisation, there seems little point in combining dextrose and amino acids in hypocaloric amounts. If a central line is felt to be necessary, then it would seem sensible to administer hypertonic dextrose solutions to provide a full calorie requirement by intravenous hyperalimentation. There may however be some merit in using slightly more concentrated amino acid solutions than the

3 - 3.5% solutions in common use if it is decided to give more amino acid as suggested by Freeman's group who found improved nitrogen balance with 1.7 gram nitrogen per kilogram body weight than with 1 gram per kilogram (115).

Nitrogen balance has its limitations as a parameter of nutritional improvement. In starvation there is a gradual decline in the excretion of urinary nitrogen as adaptation to starvation ketosis progresses. Malnourished people may therefore adapt sooner and have better nitrogen balances for a given intake of protein. Once nitrogen is supplied in fasting it may take some days before this is reflected in the urinary nitrogen with adjustment to the new nitrogen equilibrium. The nitrogen balance therefore improves then becomes worse again. Nitrogen balance is only a measure of the difference between intake and output. It measures neither protein synthesis nor protein catabolism and it cannot differentiate between nitrogen held in non-protein pools or that utilized for protein synthesis. It is only by the use of techniques in which net tissue synthesis is measured that the value of protein sparing therapy can be confirmed.

Two studies do provide evidence of the benefit of this nutritional technique. That of Skillman et al, in which an enhanced albumin synthetic rate was found in the patients given amino acids, does suggest that at least one aspect of what can be broadly classed as "visceral synthesis" is improved by a regular supply of amino acids after surgery (97). Body composition studies which Shizgal has reported also demonstrate the value of protein sparing therapy (116). Two groups were studied, one given glucose alone and the

other a casein hydrolysate. By the fifth day after surgery there was a significant loss in body weight in both groups, but whereas in the glucose group there was loss from the lean body mass and of body fat, the weight loss in the protein group was entirely from body fat. Further, in the glucose group there were changes characteristic of malnutrition in that the body cell mass had contracted and the extracellular mass had expanded. In contrast in the protein group there was no change in the extracellular mass and the body cell mass had increased slightly.

The second of the studies in this thesis is designed to assess a further aspect of protein sparing therapy. It has been suggested that the retinol transport system has a high priority in the restoration of protein status in refeeding those who are malnourished (30). If the depression of the proteins forming this system after surgery is analagous to that found in the malnourished, then early rises in the serum concentrations could be expected with a constant provision of dietary amino acids. Those patients in whom no such supply of amino acids was maintained would be expected to show a continuing decline in the serum concentrations of prealbumin and retinol-binding protein. The pattern of one of these proteins, as also albumin and transferrin, whose use as markers of nutritional status has previously been discussed, was followed after surgery in groups given amino acids and given dextrose.

Given that protein sparing therapy is beneficial and is dependent on the development of ketosis, it can be suggested that the earlier after operation this adaptation can occur, the more

beneficial will be the administration of amino acid solutions. A further aim of this study is to examine whether the manipulation of the metabolic substrate environment by pre-operative adaptation to the ketotic state had any effect on the metabolic response to stress, or indeed showed any greater benefit in terms of accepted parameters than standard post-operative protein sparing therapy. Patients were fed on a carbohydrate free, protein containing diet before surgery and after their operations were maintained on amino acid solutions.

This second study was also used to elucidate what influence, if any, the provision of amino acids had on the response of the acute phase proteins to surgery both in comparison with those "ketoadapted" before surgery and those given dextrose alone.

METHODS

STUDY 1

The aims of this study were:

1. To compare the response of serum albumin and transferrin to enteral and parenteral feeding in non-stressed malnourished patients.
2. To compare the relative efficiency of these routes of nutrition in restoring these serum protein concentrations, using nitrogen balance and apparent net protein utilisation as parameters of nutritional repletion.

Patients selected were referred for nutritional therapy. The criteria for inclusion in this study were:

a) Malnutrition, as assessed by a serum albumin concentration below 3.5 g/dl or serum transferrin concentration below 200 mg/dl and,

b) Convalescence.

This latter criterion excluded any patient operated on within one week prior to the initial protein assays and any with evidence of infection as indicated by daily temperatures above 38°C and pulse rates greater than 100 per minute. An isolated spike of fever or raised pulse rate which settled within 12 hours was not considered to indicate sepsis. Further, no patient undergoing chemotherapy or radiotherapy was included in the study, nor had any patient received a transfusion of whole blood, plasma protein fraction or albumin in the week before the study or during it.

Albumin and transferrin assays were carried out as part of the

standard biochemical assessment of patients receiving nutritional therapy. Albumin was assayed (117) as one of a multiple analysis using the Technicon Instruments Corporation SMA 12/60 Autoanalyser. The analytical variation was ± 0.1 g/dl for one standard deviation. Transferrin assays were carried out by a radial immuno-diffusion technique (118), using plates supplied by Helena Laboratories. The end point was read optically. The analytical variation was ± 35mg/dl for one standard deviation. Both assays were carried out by technical staff in the Meissner Laboratories, the laboratories associated with the New England Deaconess Hospital.

Most of the patients in the group who required enteral nutrition were fed commercially available defined formula diets such as Isocal^{R} through a nasogastric tube (9.6 or 7.3 FG Keofeed^{R} tube, a silastic, mercury tipped tube) or feeding enterostomy. A few ate normal hospital diets which were supplemented with additional nutrient drinks under the supervision of the dietician who was a full time member of the Nutritional Support Service. A record of intake was maintained for all these patients from which estimates of calorie and protein consumption were made. Daily calorie requirements were set at 1.5 x Basal Energy Expenditure (BEE) (119) and protein intake for anabolism was set at 1.5 g/Kg body weight per day. At times the actual intake differed from that which was hoped for due to not only the voluntary nature of oral feeding, but also the occasional interference by diagnostic tests or treatments.

The Basal Energy Expenditure is a measure of the energy require-

ments of a fasting individual who is physically and mentally at rest. Many factors influence this of which sex, height, weight, body constitution, age and hormonal balance are the most important. Standards have usually related the BEE to the body surface area, but indirect methods for its calculation exist which involve the measurement of oxygen consumption, carbon dioxide production and nitrogen excretion. For the studies in this thesis the BEE was calculated using the well established formulae which makes up the Harris Benedict Standards (120):

For males: BEE = 66.473 + (13.7516 x weight) + (5.0033 x height) - (6.755 x age)

For females: BEE = 655.0955 + (9.5634 x weight) + (1.8496 x height) - (4.6756 x age)

Weight is expressed in kilograms, height in centimeters and age in years.

As the BEE thus calculated takes into account a number of parameters, the caloric intake expressed as a ratio of the BEE is a better estimate of intake than if the intake was solely related to the body weight alone (Kcal/Kg).

Parenteral nutrition was provided through a central venous catheter, i.e. Intravenous Hyperalimentation (IVH). The carbohydrate source was hypertonic dextrose, usually 50%, and the "protein" source was crystalline amino acid solutions. These were mixed in infusion bags under a laminar flow hood with the necessary electrolytes, vitamins and other co-factors such as calcium, magnesium, phosphate and trace metals. Variable concentrations of amino acids and dextrose were used or the

quantity delivered was varied for each patient to achieve the planned calorie requirement of 1.76 x BEE which has been shown as optimal for anabolism (121), and planned protein requirement of 1.5 g/Kg/day. Other forms of total parenteral nutrition including those with fat emulsions were not used in this study. No patient in this group was allowed any oral nutrition.

Nitrogen balances (N bal) and calorie intakes were calculated at least twice a week and at least four times in every patient during the period of the study. Nitrogen balance was calculated from the formula:

$$N_{bal} = N_{in} - N_{out}$$

where N_{in} is the total nitrogen consumed over the 24 hour period and N_{out} is the sum of the urine urea and non-urea nitrogen. The urine urea nitrogen was measured in the Meissner Laboratories using a 1:10 dilution of an aliquot from the 24 hour urine collection by an automated method using the SMA 6/60 Analyser Technicon Instruments Corporation) (122). To this figure a constant of 2 grams was added to represent the non-urea urinary nitrogen (123, 124, 125).

The relationship of protein synthesis to protein catabolism was estimated from the measurement of the nitrogen balance. The percentage of protein retained in the body cell mass was determined from a calculation of the apparent Net Protein Utilisation (NPU) by the formula:

$$NPU\ (\%) = \frac{N_{in} - (N_{out} - N_{obg}) \times 100}{N_{in}}$$

where N_{obg} is the obligatory nitrogen loss. The apparent Net Protein Utilisation was felt to be an important parameter to calculate as this would give an indication of the likely wastage of expensive nutrients if it was found to be low. The obligatory nitrogen loss is the total nitrogen loss while on a nitrogen free diet providing sufficient energy to meet normal requirements (126). The value can be approximated to 0.1 g nitrogen per Kg of ideal body weight (1).

STUDY II

Aims

The aims of this study were:

1. To compare the effect of isotonic amino acid and hypocaloric dextrose infusions after major surgery using established parameters of early nutritional repletion.
2. To assess whether partial ketoadaptation before surgery with a carbohydrate-free protein containing diet continued after surgery confers benefit over such therapy given only in the post-operative period.
3. To compare the response of acute phase proteins and immunoglobulins after major surgery in patients given protein sparing therapy with those given tranditional glucose and balanced electrolyte infusions.

SELECTION OF PATIENTS

Patients selected for this prospective study were expected to undergo major abdominal or pelvic surgery with the need for

intravenous fluids for at least four post-operative days.

The following were excluded:

Any patient deemed to be severely malnourished;

Any patient with a recent history of myocardial infarction, congestive cardiac failure or cardiac cachexia;

Any patient with diabetes mellitus requiring insulin;

Any patient with extensive extra-abdominal malignancy.

NUTRITIONAL STATUS

This was assessed by weight for height, triceps skin fold, mid upper arm muscle circumference, serum albumin and serum transferrin concentrations. If the weight for height ratio (Metropolitan Life Insurance Company tables) or arm muscle circumference was in the range of 75 - 89% of standard (WHO tables), slight malnutrition was considered to exist and below 75%, moderate malnutrition existed. As triceps skin fold has to decrease to 50% of standard before it enters the 5th percentile, it is a less accurate measure of malnutrition (127). For this study 40-50% of standard indicated slight malnutrition. In addition a serum albumin concentration of less than 3.5 g/dl or transferrin concentration less than 200 mg/dl characterised moderate malnutrition.

If a patient was $<$ 60% normal on the weight for height assessment of had a serum albumin concentration of $<$ 3.0 g/dl he was deemed severely malnourished and excluded from this study. The assays of serum albumin and transferrin used for assessment were carried out as in Study I.

EXPERIMENTAL DESIGN

It was initially planned to randomise the patients entered into the study by drawing cards prepared by a statistician which allocated the patient into one of three groups. However, as the protocol (see below) for one group (Group A) demanded that patients be given a specific diet for two to three days before surgery, and as some patients were admitted only the day before surgery, or required some pre-operative preparation which prevented their inclusion into this group, it was not possible to have the whole patient population randomised in this manner. Further it was not considered economically justified to ask that surgery be delayed.

The method adopted was to randomise the patients as far as possible. If a patient was allocated to Group A and surgery was scheduled before the pre-operative dietary manipulation could be completed, this allocation was kept for the next patient who could fit in, and a further card was drawn.

Informed consent was obtained by me from all patients in this study and also from surgeons in whose care they were.

PROTOCOL

The protocol for each of the three groups was as follows:

Group A: These patients were given a carbohydrate-free protein sparing diet with added potassium, calcium and vitamins or carbohydrate-free intravenous amino acid infusions and vitamin for two to three days before surgery. The oral diet allowed 1 - 1.5 g protein/Kg body weight per day. The intravenous infusion

gave between 2.5 and 3 litres of near isotonic (3 or 3.5%) crystalline amino acid solutions.

Urine was checked for the presence of ketone bodies every eight hours by Labstix^{R} (Ames Company).

During surgery blood loss was replaced as required. Other fluids were given as carbohydrate-free balanced electrolyte solutions (Ringers Lactate or Normal Saline).

In the post-operative period isotonic crystalline amino acid infusions were given to meet fluid requirements. Electrolytes, vitamins and co-factors (Calcium, magnesium and phosphate) were added as appropriate to each patient. Infusions were continued as long as the surgical staff considered intra-venous fluids to be necessary.

Group B: These patients were allowed a normal diet until the night prior to surgery. Operative and post-operative fluid requirements were as for Group A patients.

Group C: These patients were allowed a normal diet until the night prior to surgery. During surgery blood was replaced as required. Other intravenous fluid was given by isotonic glucose and electrolytes solutions. These solutions were, with added vitamins, continued in the post-operative period.

ORAL FLUIDS

Patients were permitted oral fluids within the period under study.

Those in Groups A and B were allowed carbohydrate-free or minimal carbohydrate containing fluids such as water, tea, coffee bouillon or sugar-free diet sodas in amounts clinically indicated. Patients in Group C were allowed carbohydrate-containing liquids. When oral intake reached 600 ml/day, the study terminated.

BLOOD AND BLOOD PRODUCTS

Apart from blood replaced during surgery whole blood or packed red cells were transfused when clinically indicated.

Colloid (Albumin or Plasma Protein Fraction) was not used except where haemodynamic needs dictated.

A record was kept of the use of these products.

ANTIBIOTICS

If these were required by the intravenous route, they were administered in normal or half-normal saline to patients in Groups A and B, and in any carrier for Group C patients.

OBSERVATIONS

a) Prior to Surgery: Age, sex, height, weight.
Group A patients only - eight hourly urine testing for ketone bodies.

b) After Surgery: Six hourly temperature
Six hourly pulse rate
Chart of fluid intake and output
Eight hourly urine testing for glucose and ketone bodies.

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LABORATORIES DETERMINATIONS

	Pre-operative	Post-operative days (Day 0 is day of surgery)						
		Day 0	1	2	3	4	5	6 7
SMA 6(i)	x		x		x			
SMA 12(ii)	x							x or x
FBC(iii)	x		x		x			
Substrate Profile(iv)	x	x		x		x		x or x
Albumin(v)	x	x		x		x		x or x
Transferrin(v)	x	x		x		x		x or x
Prealbumin(v)	x	x		x		x		x or x
Acute Phase Protein Profile(v)	x	x		x		x		x or x

(i) SMA 6 Determinations of Blood Urea Nitrogen (BUN), Glucose, Sodium, Potassium, Chloride and Bicarbonate ions.

(ii) SMA 12 Determinations of Bilirubin, Serum Glutamic Oxaloacetic Transaminase, Lactic Dehydrogenase, Alkaline Phosphatase, Uric Acid, Cholesterol, Creatinine, Calcium, Inorganic Phosphate and Albumin.

(iii) FBC Determinations of Haemoglobin, Haematocrit and White Blood Cell Count. A differential white cell count was carried out on admission to the hospital.

The above determinations were carried out in the Meissner Laborat-

ories. SMA 6 and 12 assays were run on a Technicon Instruments Corporation SMA 6/60 or 12/60 Autoanalyser and the FBC determination on a Coulter Counter.

(iv) Substrate Profile - Determinations of the blood glucose, lactate, β-hydroxybutyrate, and acetoacetate concentrations were carried out by laboratory technicians in the Nutritional Metabolism Laboratory. Plasma free fatty acid and serum insulin analysis were carried out in the laboratory of the Professor of Biochemistry at the University of Massachusetts Medical School, Worcester, Massachusetts.

(v) Albumin, Transferrin, Prealbumin and the Acute Phase Protein Profile were assayed by technicians at the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, through the assistance of Major M.C. Powanda, Ph.D.

TECHNICAL METHODS

Blood collections for (iv) and (v) were carried out between 8 a.m. and 9 a.m. on the appropriate days. 15 ml of blood was drawn and divided into a 10 ml aliquot in a tube containing sodium heparin as an anticoagulant and 5 ml in a plain tube. Both tubes were placed on ice.

2 ml of the heparinised blood was used to estimate blood glucose, lactate, β-hydroxybutyrate and acetoacetate after deproteinisation with perchloric acid using procedures taken from Bergmeyer (128). 5 ml of heparinised blood was centrifuged for 15 minutes

and the plasma separated. Plasma free fatty acids were assayed on this sample by an acidimetric titration technique after Dole and Meinerty (129).

5 ml of blood in the plain tube was allowed to clot. After centrifugation for 15 minutes the serum was separated from the cells and used for determination of insulin, and the protein profiles. Insulin was assayed by a radioimmunoassay using Soeldner and Stone's method (130).

ALBUMIN, TRANSFERRIN AND ACUTE PHASE PROTEIN PROFILE

A modification of an automated system for immunoassay by nephelometry was used to quantitate serum albumin, transferrin and α_1-acid glycoprotein (α_1 AG), α_1-antitrypsin (α_1 AT), haptoglobin (Hp), α_2-macroglobulin (α_2M), C_3 complement (C_3), γA-immunoglobulin (IgA), γM-immunoglobulin (IgM) and γG-immunoglobulin (IgG). The immunoassay was run on a Technicon Specific Protein Auto Analyser System (Technicon Corporation, Tarrytown, New York) as described by Ritchie et al (131) with the modification of dilution being carried out with a Micromedic Systems dilutor to minimise the variability of manual dilution. The accuracy of this automated system compared with radial immuno-diffusion techniques has been published by Bostian and her co-workers (94), who themselves carried out this series of assays.

Pre-Albumin (PA), Ceruloplasmin (Ceru) and C-reactive protein (CRP) were assayed using radial immunodiffusion plates supplied by Behring Diagnostics.

The serum samples sent to the U.S. Army Medical Research Institute of Infectious Diseases were kept at -28°C until analysed. To eliminate variability in antisera, all samples were analysed in one batch. Storage from 4°C, -20° and -70°C had previously been shown to have no significant effect on measurements (94).

Urine: Daily 24 hour collections were made for Urea Nitrogen and Creatinine. These latter assays were carried out in the Meissner Laboratories using a 1: 10 dilution by equivalent techniques for assay of blood urea nitrogen and creatinine on a SMA 12/60 Autoanalyser.

TERMINATION OF STUDY

Patients were withdrawn from the study if any one of the following occurred:

a) A rise in BUN greater than 20 mg/dl in 48 hours, or absolute BUN greater than 70 mg/dl.
b) Intractable hypovolaemia, myocardial infarction or congestive cardiac failure.
c) Acute renal or hepatic failure.
d) Clinical shock,
e) Requirement for intravenous hyperalimentation.
f) Failure to adhere to the intravenous infusion allocated to that patient.
g) Oral intake reaching 600 ml/day.
h) Any other reason deemed to be in the best interest of that patient.

Five patients were withdrawn. Two of these (one each in Group A and C) had inoperable malignancies (of stomach and of pancreas) for which no definitive surgery was carried out. Hence they were excluded on the grounds that major abdominal surgery was not carried out. One patient in Group B was excluded as he developed a ventricular arrhythmia for which a lignocaine infusion in dextrose was administered on the day of operation and first post-operative day. He later developed renal failure and was excluded under paras. (c) and (f). A fourth patient excluded, also in Group B, developed post-operative septic shock and renal failure and was excluded under paras. (c) and (d). The fifth patient, in Group C, had signs of early post-operative peritonitis and required a further laparotomy on the third post-operative day.

None of the data from any of these patients is included, nor is reference made to them again.

STATISTICAL METHODS

The two studies produced data which was analysed in a number of ways. In the first in which the rise of the serum concentrations of two proteins was measured in each patient in the two groups, Student's t test for paired groups was used. When the results between the groups were analysed for significant differences, the t test for unpaired groups was used. Calculations were done on a Monroe 1860 Calculator both by myself and by Lyle L. Moldawer, B.S., a research assistant in the Nutrition Metabolism Laboratory.

In the second study in which serial group data, with variable

numbers in each group was compared for three groups for a series of proteins, two-way analysis of variance with replication was carried out. Two-way analysis of variance was used to determine whether there was an overall effect due to either one of the hypocaloric nutritional regimens (row effect) or whether the passage of time produced a change (column effect). Analysis with replication was carried out when such an effect had been demonstrated to try to show with which regimen or which days differed from the others. With replication no p values to indicate the level of significance are stated in the text. Significance was only looked for at the $p<0.05$ level and, if found, it is stated that certain results "differed" from other results. The method is described by Snedecor and Cochran (132).

Further analysis was carried out within each group and between each group where two-way analysis of variance had shown significance by t tests for paired and unpaired groups as relevant. Such tests are referred to in the text.

The Prophet System (Chemical/Biochemical Information-Handling Program of the National Institute of Health, U.S.A.) was used as the basis for the computer run two-way analysis of variation with replication. This was carried out by Hugh Y. Rienhoff, B.A.
T tests were done as above on the Monroe 1860 Calculator.

RESULTS

STUDY I - TRANSFERRIN AS A MEASURE OF THE EFFICACY OF HYPER-ALIMENTATION

CLINICAL CHARACTERISTICS

Twenty patients satisfied the criteria for inclusion in the study. Ten had been given enteral feeding (ENT group) and ten intravenous hyperalimentation (IVH group). Their clinical particulars, serum albumin and transferrin levels, are charted in Table 1. There were five males and five females in the ENT group, and six males and four females in the IVH group. There was no significant difference in age between the groups.

Basal Energy Expenditure (BEE), as calculated using the formulae from the Harris-Benedict Standard which has been discussed earlier, ranged from 948 to 1921 Kcalories (mean 1407 $\pm$ 308 Kcalories) in the ENT group and from 1097 to 1898 Kcalories (mean 1419 $\pm$ 267 Kcalories) in the IVH group. This derived parameter was not different on statistical analysis by t testing between the groups.

The ENT group was studied for a mean of 15 days (range 12 - 19 days) over which time there was a mean loss in weight of 1.0 Kg (range +2.8 Kg to -4.6Kg). The IVH group had a mean of 12 days nutrition (range 8 - 16 days) with no change in weight (range +3.9 Kg to -3.0 Kg). There was neither significance in the loss of weight in the ENT group, nor in the difference between the groups.

ALBUMIN AND TRANSFERRIN

The initial, final and change in serum albumin and transferrin levels are detailed in Table 2. The mean albumin concentration for the group fed enterally was 3.1 ± 0.3 g/dl prior to the study and 3.2 ± 0.3 g/dl afterwards. The corresponding concentrations for the IVH group were 3.5 ± 0.6 g/dl and 3.5 ± 0.4 g/dl. Whereas there was effectively no change in the albumin concetrations, there were rises in both groups in transferrin concentrations. In the ENT group the mean concentration rose from 152 ± 44 mg/dl to 216 ± 60 mg/dl with a similar rise in the IVH group from a mean of 138 ± 39 mg/dl to 206 ± 44 mg/dl. There was no statistical difference between the two groups for either protein, nor within the groups for albumin changes, but the increase in transferrin concentration within each group was highly significant, $p < 0.001$.

NUTRITIONAL INTAKE

The caloric intake in the ENT group varied from 1496 Kcalories to 2365 Kcalories per day (mean 2077 ± 248 Kcalories). It is accepted that these intakes can only be an estimate and that giving them to an accuracy of four figures is perhaps seeking after inappropriate perfection, but they are given in the knowledge that an honest attempt was made to document the total intake of these patients. It was much easier to calculate the intake in those patients, the majority, in whom nasogastric feeding by defined formula diets using a pump was the route of nutrition, as the calorific value and the protein content of the solutions were specified. The actual intake often differed from that planned due to individual patient acceptance of food, particularly

if nausea or a bloated feeling developed with tube feeding. This would lead the pump to be switched off for a period of some hours and then restarted. Occasional breaks in feeding were necessitated by diagnostic or other investigations being carried out at the instructions of the Physicians or Surgeons in whose care the patients were.

With tube feeding the concentration of the "feed" was often reduced and the rate reduced when this feeding was initiated. This was a further factor which, though of practical value in the preparation of the gut in handling increasingly hyperosmolar loads, sometimes led to a smaller intake than would have been wished. The length of each individual study was determined as that between measurements of the proteins albumin and transferrin. Over this period the intake was averaged for each patient. This may also account for a few of the patients apparently being fed for periods of less than the two week period under study.

In the IVH group the daily range of intake was 1974 Kcalories to 3246 Kcalories (mean 2635 $\pm$ 366 Kcalories). This intake was likely to be more accurately assessed than for enteral nutrition as it involved the calculation from infused volumes of known composition. The most likely error was the inaccurate recording of the volume infused by nursing staff, and the changing of bags of fluid before they were completely empty. Nevertheless, most nurses co-operated with interest and enthusiasm once the relevance of accurate recording was explained to them.

To relate the calorie intake to the patients' needs the ratio of

of this to the Basal Energy Expenditure was calculated. In the ENT group the range was 1.10 to 2.46 x BEE (mean 1.53 ± 0.36 x BEE). For the IVH group, as with calorie intake, the range was higher overall, 1.56 to 2.42 x BEE (mean 1.88 ± 0.25 x BEE). For both calorie intake and for the Calorie:BEE ratio there was a significant difference between the groups, $p < 0.001$ for calorie intake and $p < 0.02$ for the Calorie:BEE ratio.

There was, however, no significant difference between the two groups' average daily nitrogen intake. The ENT group was given a mean of 14.0 ± 3g (range 8.9 to 18.2g). The IVH group received a mean of 14.9 ± 2.8g (range 9.4 to 19.5g). These figures correspond to a protein intake of 1.5 ± 0.3g/Kg body weight (range 1.0 to 1.9 g/Kg) in the ENT group and 1.6 ± 0.5 g/Kg body weight (range 1.0 to 1.9 g/Kg) in the IVH group.

Within the groups there was a wide variation in the nitrogen: non-protein calorie ratio (N:Cal), in the nutrition received. For the patients on IVH the range was 1:107 to 1:327 with a mean of 1:162, whereas for the ENT group the range was 1:85 to 1:219 with a mean of 1:133.

NITROGEN BALANCE

In the patients fed through the enteral route the mean nitrogen balance was +3.0 ± 2.8 g/day with a range in individual patients of -2.0 to +7.2 g/day. A valid criticism in the assessment of nitrogen balance might be that no measurement was made by any accepted method such as a Kjeldahl technique for faecal losses. This would be particularly relevant for this group, as opposed

to the group given intravenous hyperalimentation . However it was long standing practice in the laboratory, based on some unpublished work and supported by Kaminski (125) that in patients fed either by defined formula diets or by intravenous hyperalimentation, there was a loss of 2 grams of nitrogen per day to account for the urinary, non-urea nitrogen losses together with that of skin. This accounts for loss of ammonium ion and nitrogen in uric acid, creatinine and amino acids. By the addition of this standard figure to each day's output of nitrogen for each patient, it was felt that this would average out the losses, rather than have a loss recorded on the days on which faeces was passed, particularly as patients being fed on these low residue diets tend to have relatively fewer bowel motions than those on normal diets.

The patients on intravenous hyperalimentation had a significantly higher mean nitrogen balance of $+4.3 \pm 1.9$ g/day with an individual range of +1.8 to +6.7 g/day ($p < 0.01$).

Regression lines were drawn by plotting the nitrogen balance for each day against the Calorie:BEE ratio for that day (Figure 1). Nitrogen equilibrium with enteral feeding was achieved at 1.21 x BEE and for intravenous hyperalimentation this was at 1.55 x BEE Using Armitage's technique to adjust for errors in prediction in regression analysis (133), the calorie intake at which nitrogen balance would be achieved with a 95% confidence limit was 1.34 x BEE for enteral feeding and 1.70 x BEE for parenteral nutrition. The calorie intake which these figures represent, and therefore which can be considered to indicate the intake for

anabolism to be certain to occur, are 41.5 Kcal/Kg for intravenous hyperalimentation and 33.3 Kcal/Kg for the enteral route.

APPARENT NET PROTEIN UTILISATION

The mean apparent NPU for the ENT group was 62 ± 9% while receiving 1.53 x BEE (1407 ± 308 Kcalories/day) and 73 ± 10% in the IVH group receiving 1.88 x BEE (1419 ± 267 Kcalories/day). Figure 2 shows the regression lines for the two groups. At the point of nitrogen equilibrium with enteral feeding (1.21 x BEE), the NPU was 60%, while the value for IVH at this calorie input was 35%. Nitrogen equilibrium with parenteral feeding occurred at 1.55 x BEE where the NPU for enteral and parenteral feeding was 66% and 47% respectively. These figures suggest a greater efficiency of enteral feeding when expressed in terms of apparent net protein utilisation.

STUDY II - NUTRITIONAL MARKERS AND ACUTE PHASE PROTEINS IN KETOADAPTATION, PROTEIN SPARING AND DEXTROSE THERAPIES

CLINICAL CHARACTERISTICS

Twenty-four patients completed the study, seven in Group A, nine in Group B and eight in Group C. The age, sex, diagnosis and operation carried out for each patients is recorded in Table 3. This mean age for patients in Group A was 56.1 years, for those in Group B 61.8 years and for Group C 59.0 years. By Duncan's Multirange test the groups can be considered similar. The surgery performed was considered to be equivalent in operating time between the groups.

The nutritional parameters of weight for height, triceps skin fold and arm muscle circumference, all as percentage of standard, and the initial serum albumin and transferrin concentrations are recorded in Table 4 with the nutritional status assessed as stated under "Methods".

Group A had one moderately malnourished patient, Group B had four malnourished patients, of whom two were moderately malnourished and Group C had three malnourished patients, one moderately so. Thus prior to surgery 33% of the patient population of this study were assessed as having some degree of protein-calorie malnutrition. The criteria by which these patients were assessed illustrate the difficulty of using only one parameter for definitive classification of degrees of malnutrition. Patients AG in Group B is classed as slightly malnourished only on the basis of an arm muscle circumference of 89%, yet had this

been 1% greater, well within the limits of measurable error, and by all other criteria, he was normally nourished. Overall assessment by anthropometric means, particularly by the triceps skin fold, is probably of less validity in terms of protein malnutrition than the serum protein concentrations. Two patients in Group C were probably classified lower than necessary on the basis of their triceps skin fold measurement.

INTAKE OF NITROGEN

As the timing of surgery was variable in that some operations were carried out early in the morning and others in the afternoon, initiation of the post-operative amino acid infusions varied within the groups, as also did the collection of urine. For this reason the recordings both of nitrogen intake and urine nitrogen excretion for Day 0, the day of operation, are not presented. Day 1 is taken from 0700 hours on the morning following surgery for a 24 hour period.

The mean nitrogen intake for Group A patients was 11.7 $\pm$ 2.4 g/day with an individual range from 8.7 to 14.8 g/day. The protein equivalent gave a mean of 74 $\pm$ 16 g/day (range 54 to 94 g/day) or 1.0 $\pm$ 0.3 g/Kg/day (range 0.5 to 1.4 g/Kg/day). Figure 3 shows the daily mean intake for Groups A and B. For Group B patients the mean intake was 12.9 $\pm$ 2.8 g nitrogen/day (range 7.0 to 16.8 g/day) or protein equivalent of 81 $\pm$ 18 g/day (range 44 to 107 g/day). Intake in terms of weight was 1.3 $\pm$ 0.5 g/Kg/day (range 0.7 - 2.2 g/Kg/day). There was no statistical difference between the intakes in Groups A and B by Student's t test for unpaired groups.

Group C patients received no regular nitrogen intake at all. However in patients in all groups it had to be accepted that if in the period of surgery or immediately afterwards there were requirements to infuse protein products for haemodynamic support, then this would be accepted. Table 5 lists the albumin or purified protein fraction given to patients. A total of 175 grams were infused in Group A patients, 252.5 grams in Group B patients and 225 in Group C patients. However after Day 1 none of these products were used in Group A patients, only 12.5 grams in one patient in Group B, but 137.5 grams to two patients in Group C. No patient was given a blood transfusion during the course of the study except during surgery and immediately following, while in the recovery area where many of the patients who had undergone very major surgery stayed the night. The infusion of these protein products was not included in the individual nitrogen intakes, nor balances. It was regretted that any was used after the initial surgery, but this exemplified the difficulty of carrying out research outwith a metabolic unit in patients for whom members of the surgical team in whose care they were would rewrite intravenous orders despite having agreed to the format of the study.

It is appreciated that some patients received less nitrogen than would have been considered as optimal (54, 113), 1.0 - 1.7 g/Kg/day, but the fluid restrictions which were so often imposed on the elderly patients limited the volume infused. A further factor which reduced that prescribed on the fluid order chart was the practice of some of the surgeons of giving their patients routine antibiotics. These were infused intravenously in a small quantity

of normal saline or dextrose as mentioned earlier. This reduced the volume of fluid available for the infusion of amino acid solutions.

NITROGEN BALANCE

The mean daily nitrogen balance (Table 4) and the cumulative nitrogen balance for each group are recorded in Table 6. Over the first seven days following surgery the net loss in Group A was 37.4 grams, in Group B this was 25.5 grams and for the patients in Group C the mean loss was 70.2 grams over the same period. In all groups the daily nitrogen balance tended to stabilise around day 5 at which time it would be expected that the response to injury would be established in the adaptive or flow phase. There was a much improved nitrogen balance both on a daily and cumulative basis in the two groups given amino acids which was confirmed by two-way analysis of variance with a p value of < 0.001. Analysis with replication confirmed that these two groups were different from Group C, but not different from each other. No significance was attributed to the passage of time. Figure 4 illustrates that a changing pattern was not evident for Group A or B although the trend in Group C was for the nitrogen balance to improve after surgery to day 5, thereafter remaining the same.

SUBSTRATE PROFILE

The energy substrate profiles of blood glucose, lactate, ketone body (the sum of β-hydroxybutyrate and acetoacetate) and plasma free fatty acid concentrations are recorded in Table 7 together with serum insulin concentrations. All groups showed a response

to surgery, i.e. a change from the pre-operative values, and in Groups A and B the pattern of developing ketoadaptation after surgery was evident.

The blood glucose concentration (Figure 5) in each group changed as a result of surgery. There was a statistically significant effect of time and also of nutritional influence throughout the study with a p value of < 0.001 for both parameters. The pre-operative concentration in Group A is lower (though not statistically so by t testing) at 4.56 mM, than the means for Groups B and C, 6.22 mM and 6.40 mM respectively. The latter two reflect an overnight fast whereas that for Group A is consistant with a period of glucose-free feeding before surgery. The immediate post-operative rise in glucose concentrations were similar in Groups B and C, with a higher peak in Group A. Thereafter the concentrations in Group B tended to approximate to those in Group A with those in Group C remaining higher. Analysis with variance confirmed that Group C results were different from those of Group A and B and that there was a statistical difference in the overall post-operative concentrations when compared not only with those before surgery, but also on all other post-operative days, emphasising that the surgical procedure initiated these changes.

Of note is that in Group A the blood glucose concentration had fallen by day 2 to the same level as before surgery and that thereafter there was no further fall. In Group B the concentration fell to below that found before surgery by day 2 and remained low, but no lower than in Group A. In Group C a lower concentration

was found only on day 4, but otherwise tended to be slightly above that after an overnight fast, almost certainly a feature of the constant infusion.

The blood lactate concentrations (Figure 6) also show a statistically significant effect on time ($p < 0.01$) and nutrition ($p < 0.05$). As with glucose, the immediate post-operative values are different from all other days. The lower concentration pre-operatively for Group A indicates a reduction of carbohydrate precursors, understandable on the basis of a glucose-free diet. Even by analysis with replication no single group was shown to be different from the others.

It is unclear why the pre-operative concentrations in Groups B and C should be so different as both were measured after an overnight fast. They remained much higher than the mean for Group A immediately after surgery but in all groups the concentrations were similar by the second day after surgery and this was maintained.

Plasma free fatty acid analysis (Figure 7) and total blood ketone bodies (Figure 8) confirm that in Group A, in whom the mean concentration was very considerably elevated before surgery, there had been some adaptation to the use of fats as the main energy source, even after only two days of a carbohydrate free diet. Overall the effect of surgery, time ($p < 0.05$) and a nutritional component ($p < 0.05$) were all shown to have significant effects on the free fatty acid concentrations. The immediate post-operative levels were different from all other days. Group C was

shown to be different from Groups A and B by analysis of variance, but these two latter were not different from each other.

It is however worth noting that in Group A patients there was hardly any difference in the pre and post-operative concentrations, 880 and 897 mEq/l. The post-operative mean in Group B was comparable to that in Group A, as indeed was that for Group C, but after surgery the levels in Groups A and B were comparable on all the other days, whereas in Group C there was a much lower mean concentration from day 2 onwards.

The blood ketone body concentrations (Figure 8) were also changed by surgery and the differing nutritional regimens ($p < 0.001$). As with free fatty acids, Group C was different from the other two groups receiving amino acids, but these two groups were also shown to differ from each other by analysis with replication. No significance could be attributed to the passage of time.

A probable explanation for this latter observation is seen from close examination of the figures. There was hardly any change in Group C concentrations. Those of Group A were already elevated before surgery due to the development of the ketotic state. There was a slight fall after surgery and only by day 6/7 did the concentration become raised. The pattern in Group B was very different, which would account for the groups differing by analysis of variance in that the initial low concentration (0.08 mM) rose slightly after surgery, was maintained for two days, but by the fourth post-operative day had risen markedly, a trend

continued to day 6/7.

Insulin concentrations are also recorded in graphical form (Figure 9). The pre-operative mean for Group A was 16μunits/ml, which was higher than for Group B 13 μunits/ml and Group C 10 μunits/ml. In each group there was a rise after surgery, after which in Group C there was a further rise followed by a fall towards the concentration found in the immediate post-operative period. In the other Groups the concentration fell consistantly with a levelling off by days 4 and 6/7 to pre-operative levels. The differing nature of Groups A and B from Group C was confirmed by two-way analysis of variance with replication ($p < 0.01$). There was an overall significant effect of time ($p < 0.05$) with the immediate post-operative results different from those before surgery and on all other days.

ACUTE PHASE PROTEIN PROFILES

The mean and standard deviation for the concentration of each of the proteins in each of the groups for each of the days under study are recorded in Table 8. Unlike the substrate profiles in which there was a different group there was no such difference noted with the acute phase protein profile. All three groups were very similar, which would be expected as in none of the groups had there been any specific stimulus given to initiate an acute phase response. The results were analysed by two-way analysis of variance with replication to determine the overall effect of time or nutritional influence. Within groups t testing was carried out to compare the mean concentration on each day with that before surgery.

α_1-Acid Glycoprotein (Figure 10) results did not show any effect due to the differing nutritional regimens. There was, however, some significance due to time ($p < 0.01$) although analysis with replication did not indicate where. There was a rise in concentration peaking around days 4 and 6/7, but whereas in Groups B and C this was outwith the upper limit of normal, the rise in Group A was much more modest, 29 mg/dl, as contrasted with 57 mg/dl in Group B and 40 mg/dl in Group C. The rise in Group B on these two days was of significance at the $p < 0.05$ level, but this was not observed for Group C, despite a similar peak level, presumably because the initial level was somewhat higher. The pattern in Group A was much more of a gradual rise as seen in Figure 10.

α_1-Antitrypsin Concentrations (Figure 11) paralleled those of α_1AGP in the effect of time ($p < 0.01$). In both Groups B and C there was an initial drop over the time of the operation and then a rise which had peaked on day 4. In these two groups there was virtually no difference between the recorded concentrations on any day whereas the pattern in Group A was of a gradual rise to a peak on day 4 which was much lower than for the other two groups, 457 mg/dl as compared with 566 mg/dl and 565 mg/dl in Groups B and C. By analysis with replication, the pre-operative concentrations were statistically different from that on days 2, 4 and 6/7, as also was the post-operative concentrations. This increase was also significant within Groups B and C on those days, but there was no significance in the increase within Group A on any day. No nutritional overall effect was demonstrated by statistical analysis.

Haptoglobin concentrations showed significant changes in time after surgery (Figure 12) with an initial post-operative fall followed by a rise, peaking around day 4 to 6/7 ($p < 0.05$). As with the two previously reported acute phase proteins the pattern in Groups B and C was quite different from that of Group A in which there was hardly any change at all in the concentration except for a rise on day 4 of some 60 mg/dl which had returned to the pre-operative level by day 6/7. With t tests comparing the changes within each group, the post-operative fall in concentration was significant in Groups B and C at the $p < 0.05$ level as also the rise by day 4 when related to this same initial level. The rise in Group C on day 6/7 was also of signiciance at this same level, but not for Group B. No nutritional effect was established.

C_3 Complement (Figure 13) is also known as B_{1C} globulin. This was significantly affected by both time ($p < 0.01$) and nutritional influence ($p < 0.01$). As with the other acute phase proteins, α_1AGP, α_1AT and Haptoglobin, Group A results appeared to differ. Indeed by analysis with variance Group A was shown to be different. The protein concentrations tended to show a fall after surgery with only a gradual rise thereafter with the highest values recorded for Groups B and C on day 6/7. By analysis with replication the difference between the pre-operative, post-operative and day 2 data was different from that on days 4 and 6/7. However with each group there was no significant change from the pre-operative concentration by t testing. The noted nutritional influence may well have been due to the virtual absence of any change in the Group A concentration over the first two days after surgery,

although with unpaired t testing between the groups the baseline concentration in Group A was higher than in Group B ($p < 0.05$) but not than Group C.

C Reactive Protein concentrations (Figure 14) did rise significantly after surgery and hence time was shown to have an effect ($p < 0.01$). In all groups there was a peak on day 2 which was subsiding by the end of the week. The overall concentrations on days 2 and 4 were higher at least at the $p < 0.05$ level than on all other days. All groups, including Group A, showed a significant increase from pre-operative concentrations by day 2, but only in Group B was there continued significance at the $p < 0.05$ level on days 4 and 6/7. As with the other acute phase proteins except C_3 there was no discernible nutritional influence exerted.

α_2-Macroglobulin (Figure 15) was neither affected by the intravenous infusions nor the passage of time. The concentrations in all groups remained virtually the same throughout the study.

Ceruloplasmin concentrations (Figure 16) on visual inspection appeared hardly to change over the period studied in any group. This was confirmed by statistical analysis.

No correlation was found between the blood ketone body concentration and the pattern of any of the acute phase protein concentrations.

IMMUNOGLOBULIN PROFILES (Table 9)

The immunoglobulins, although not acute phase proteins, are

reported as the automated analysis included them. The effect of time was shown to be of significance ($p<0.05$) for each of IgA (Figure 17), IgM (Figure 18) and IgG (Figure 19), using two way analysis of variance. Nevertheless within all three groups and throughout the study all the values were well within the very broad limits of normality. The pattern for IgA was of an initial drop in concentration by the second post-operative day with a rise by day 6/7 which in all groups was beyond the baseline level. For IgM there was very little change at all except for a tendency to rise by the end of the week. The pattern for IgG was of a decline with restoration towards the initial level by the end of a week, which was more pronounced in the groups receiving amino acids.

Analysis with replication and t testing were of little additional help in the derivation of useful information from the data obtained. Overall for IgA there was a significant change from the results on day 2, on which the lowest values were recorded, compared with day 6/7 on which the highest results were obtained. No equivalent overall difference was found by this method of analysis for the other two immunoglobulins.

The varying nutritional regimens did play some part in the slight differences between the groups for the IgA profiles ($p<0.05$) and the IgM profiles ($p<0.001$). Analysis with replication was unhelpful in indicating where this effect could be pinpointed. For IgM it is possible that the higher concentration in Group B which was maintained throughout the study and which rose more than in the other groups may have given a false influence to this

statistic. It is difficult to guess where the influence may have been in the IgA profiles. It should be emphasised that by analysing the data by the method of analysis with variance any increase or decrease over the whole of the study within the whole population are taken into account and "comment" is made on the probability of various changes occurring by chance.

In summary, it is doubtful from the data obtained from the immunoglobulin assays that any real change occurred after surgery. Perhaps there was a dilution effect, but certainly from the occasional change considered to be of statistical significance it is doubtful whether there would be any value in considering the immunoglobulins as useful indices in the assessment of protein sparing therapy.

NUTRITIONAL MARKER PROFILES

The plasma proteins, albumin, transferrin and prealbumin were analysed in a similar way as the acute phase proteins and immunoglobulins. The serum concentrations are recorded in Table 10, and the graphical representation of each are presented in Figures 20, 21 and 22.

Albumin concentrations (Figure 20) are known to be affected by surgery. However in Group A the mean concentration was maintained above the lower limit of normal throughout the week of study, starting at 4.1 g/dl and dropping to 3.7 g/dl on day 2, but no further and plateauing at 3.8 g/dl thereafter. In Group B the pattern, an early decline from 3.9 g/dl to 3.2 g/dl was maintained as around this lower concentration for the rest of the

week, whereas in Group C, the group in whom there was no nitrogen intake, there was a steady decline from 3.9 g/dl to 2.9 g/dl over the week.

Analysis by two way analysis of variation indicated that there was an effect of time ($p < 0.001$) and also for the intravenous nutritional regimens ($p < 0.001$). The overall analysis with replication demonstrated that there were differences between the group mean concentrations on the pre-operative assay and those on days 2, 4 and 6/7, and also between the immediate post-operative concentration and these of days 2, 4 and 6/7. There was no difference between the pre and immediate post-operative concentrations for the study subjects as a whole. By paired t tests the fall in concentration was not of statistical significance in Group A, but for Groups B and C the fall from day 2 onwards was significant at the <0.05 level. Unpaired t tests to show differences between the groups demonstrated that Group A maintained the albumin concentration better than Group B ($p < 0.01$) and than Group C ($p < 0.05$) and that there was no difference between the Groups B and C.

Transferrin concentrations (Figure 21) dropped in all groups. Yet again in Group A this was much less than for the other groups, 249 mg/dl to 231 mg/dl by day 4 but then there was a further drop to the lower limit of normal at 200mg/dl by day 6/7. In Group B, there was an early drop from 244 mg/dl to 190 mg/dl by day 2, with only a slight decline thereafter. Group C concentrations showed a similar pattern to that of albumin, with a gradual decline to day 4 from 262 mg/dl to 199 mg/dl, but then a rise to 234 mg/dl

by day 6/7 which was unexpected.

There was significance both from the nutritional effect ($p < 0.05$) and also from the effect of time ($p < 0.05$). By analysis with replication overall pre-operative concentrations were different from those of days 2, 4 and 6/7. More precise analysis with t testing within the groups showed that for Group A there was a fall in mean concentration which only became significant at the $p < 0.05$ level on day 6/7. In Group B concentrations were significantly lower than those prior to surgery on days 2, 4 and 6/7. In Group C the fall was significant from the pre-operative concentration only on days 2 and 4.

Although no difference was found between the groups by analysis with replication, unpaired t tests demonstrated a similar trend to that found with albumin. Group A concentrations were better maintained than those of Group B ($p < 0.02$), but not than Group C. Group C was also better than Group B in this respect ($p < 0.05$) reflecting the unexpected observation of the rise in Group C of transferrin concentrations on day 6/7.

Prealbumin concentrations (Figure 22) showed a somewhat similar pattern to tha other two "marker" proteins. In all groups there was a decline in the mean concentration. In Group A the drop was between the end of surgery and day 2, from 16.0 mg/dl to 10.8 mg/dl, which only dropped slightly by day 6/7 by a further 1.0 mg/dl to 9.8 mg/dl. In Group B there was an initial fall of greater amount by day 2 from 15.8 mg/dl to 8.5 mg/dl and thereafter not too great a further fall, dropping to 5.9 mg/dl by day 4 and

achieving a slight rise by the end of the week to 6.7 mg/dl. The figures for Group C were somewhat similar in pattern to Group B with an early profound drop from 20.8 mg/dl to 11.3 mg/dl on day 2, a slight further drop in concentration to 9.9 mg/dl on day 4 and rise to 11.4 mg/dl on day 6/7.

Time and nutrition were both of significance with p values of < 0.001 and < 0.05 respectively. As with albumin there were differences between the overall pre-operative concentrations and those on days 2, 4 and 6/7 as also the post-operative concentrations and days 2, 4 and 6/7, but not, as indeed would be expected on viewing the results, between those before and those immediately after surgery. T testing within the groups confirmed this statistic as in each group there was a significant fall from the pre-operative concentration on days 2, 4 and 6/7 at a p value of at least < 0.05. As with the other "marker" proteins a nutritional effect had been determined. By t testing between the groups prealbumin concentrations were better maintained by Group A than Group C ($p < 0.05$), but not any better than those in Group B, nor was there any difference between Groups B and C. This finding would seem to contradict the results as displayed graphically as the concentrations in Groups A and C are very similar on days 2, 4 and 6/7. The explanation may be that as the initial concentration in Group A was lower than in Group C, the fall was less and hence the concentration overall was less disturbed throughout the week than in Group C. This observation is consistant with that of the other proteins, albumin and transferrin.

DAYS OF FEVER

The number of days during the period studied on which each patient had a temperature of 38°C or higher was recorded. The mean for Group A was 2.6 days per patient, for Group B 2.4 days per patient and Group C had a mean of 4 days temperature elevation per patient. There was no statistical significance in the difference.

Fever is not necessarily the result of bacterial or viral infection. Three patients in Group A and four in each of Groups B and C were started on a course of prophylactic antibiotics before, during or immediately after surgery and as such a potential rise in temperature might have been masked or prevented.

Where temperature elevations persisted, appropriate chest X-rays and cultures of sputum, urine or from the wound were taken. The results are summarised in Table 11. Despite equivalent numbers in each group receiving antibiotics, there was radiological or bacterial evidence of a cause for pyrexia in only one patient in Group A, two in Group B and four in Group C.

No correlation has been looked for between days of fever and any of the acute phase proteins.

DISCUSSION

Until the mid 1970s nutritional needs of patients in hospital were often neglected. This neglect can be of especial concern during illness in which a normal intake of food is impossible, precisely the type of situation in which surgery may be required. Nutritional therapy is therefore worth considering even after a few days of semi-starvation or starvation, if only in the form of supplements to a "normal" diet. Substitution by tube feeding with a defined formula diet or by one of the techniques of parenteral nutrition may be necessary, and, if so, this should be regarded as the primary route of nutrient intake until normal nutrition can be tolerated. Such therapy should be directed towards restoration of the body cell mass, the most metabolically active compartment of the body (39). Unfortunately, this cannot be done with the specificity with which, for example, antibiotic therapy may be directed at a bacterial infection. Nevertheless, the intake of adequate good quality protein is accepted as being the most essential feature.

The host defence mechanism is of importance in terms of survival, particularly from the effects of infection. This is a field in which very complex inter-relationships exist, and although it is perhaps presumptious to generalise, there is agreement that protein and calorie malnutrition is associated with some impairment of B and T cell mediated immune function (134, 135). Improvement in various immune parameters after nutritional repletion has been described, in particular the return of delayed hypersensitivity in cancer patients undergoing chemotherapy (136).

The whole field of nutrition and cancer is wide and fundamental questions such as the relative utilisation of nutrients by host or by tumour cells, or the ethical considerations of using costly treatment to support patients with widespread metastatic disease require to be answered. One early report has certainly suggested that appropriate nutrition may not only support the host, but decrease the utilisation of certain amino acids by tumour cells (137).

Protein administration alone does not meet the requirements for either maintenance or restoration of the body tissues to normal. Anabolism only occurs when there is an adequate supply of dietary energy. There is good evidence that in the malnourished this is in the range of 45 - 50 kCal/Kg when supplied by intravenous hyperalimentation (116, 138). There is less agreement about the optimal ratio of nitrogen to energy supplied. Recommendations range from 1:120 - 1:200 (39, 139) with Chen and his colleagues concluding that for complete utilisation of 1 gram of nitrogen 450 calories were required (140). Smith et al. have shown that malnourished patients can tolerate increases in the protein content of the supplied nutrient regimen to a ratio of 1:100 without loss of utilisation (141), and suggests that in those who are already malnourished there is a greater avidity to utilize amino acids than in those normally nourished.

NUTRITIONAL MARKERS IN UNSTRESSED PATIENTS

In the first study of this thesis the energy relationships of intravenous hyperalimentation and enteral nutrition were studied together with the effect of these nutritional therapies on two of

the proteins considered to be markers of nutritional repletion. It was decided to eliminate as far as possible the likely variable factors of sepsis, surgery or infusions of albumin or plasma protein by the strict selection of patients.

Weight change and nitrogen balance are often considered acceptable criteria by which to assess nutritional therapy, but they have their limitations. An increase in weight can result from fluid shifts with retention of water, changes in lean tissue or in fat or in combinations of any of these three. Neither group in this study showed an overall gain or loss in weight, although individually there were recorded changes. The data presented is that at the beginning and end of the study and intermediate changes have not been presented. Yet it was of interest to note how many of the patients had an early weight gain, which for some was lost by the second week. This refeeding oedema is often found and has been ascribed to an osmotic effect. However the gradual replacement of excess extracellular water by intracellular or indeed extracellular protein will not necessarily lead to any immediate increase in weight.

A positive nitrogen balance may indicate increased protein deposition in muscle and/or visceral organs and/or the skeleton, but cannot determine the proportional amounts nor the flux through the pool of available amino acids. Protein repletion in the malnourished will proceed along certain pathways, the priorities of which are not understood, but it is assumed that they will be adapted for "survival value". As such they may be very different from the priorities during period of stress. Because much of

protein synthesis takes place in the liver, it was felt that two proteins secreted by the liver might reasonably be used as indices of this aspect of anabolism. Albumin and transferrin were chosen because of their acceptance as nutritional indices in children.

In this study transferrin proved to be a more sensitive index of nutritional repletion than albumin as there was a significant increase in the serum concentration ($p < 0.001$) in both groups fed either enterally or parenterally, whereas no increase in albumin concentrations were recorded. This finding in adult patients with mild to moderate malnutrition supports that of McFarlane and his colleagues (18) and also that of Reeds and Laditan (142) who advocated that role of transferrin in monitoring the effects of refeeding in children.

The value of using transferrin in this context as a nutritional marker has been discussed earlier. Young and Hill have fairly recently, in 1979, established the validity of transferrin within the context of protein calorie malnutrition by the use of a complex matrix of bivariate correlation for data which included many possible parameters of malnutrition (47). Transferrin, and also prealbumin, showed such a correlation with many of the other variable indices that some mutual dependence on common factors was suggested. Inevitably protein calorie malnutrition was considered to be one of these factors, with the protein concentrations reflecting the reduced protein turnover associated with malnutrition.

The concentration of a protein in the serum is dependent on a number of inter-related factors: the rate of synthesis and

release, the rate of catabolism, utilisation, the rate of complexing and removal of the complex, the distribution between the intra-vascular and extra-vascular compartments and the equilibration rate, the degree of hydration and tissue permeability. The overall balance between these physiological processes determines whether the concentration of any particular protein reflects the overall increase or decrease in protein turnover.

In this study the assumption is made that net protein synthesis took place and is justified by the positive nitrogen balance which was evident for both groups. Despite the better nitrogen balance which was found with intravenous hyperalimentation, with only a very marginally greater intake of nitrogen, the rise in the transferrin concentration was similar in both groups. Some comments will be made on the relationship of nitrogen balance to the method of nutrition in a subsequent paragraph, but it should be emphasised that as the transferrin concentration was the only parameter to change after feeding, other than nitrogen balance, it can be considered to be a most useful index of nutritional repletion. Its value may be particularly relevant in hospital practice where hypoalbuminaemic patients may be infused with albumin, thus distorting the value of albumin as a nutritional index.

The effect of protein deprivation and refeeding on albumin and transferrin synthesis have been discussed earlier with mention of the greater sensitivity of albumin synthesis to dietary protein supply than that of transferrin synthesis (10). In malnourished

man with reduced albumin concentrations, the absolute catabolic rate of albumin is lower than normal (8). Less is known about the dynamics of transferrin synthesis and catabolism. The extra-vascular/intra-vascular ratio of albumin is decreased in malnutrition (8), possibly a mechanism by which maximal plasma protein concentrations are achieved. During refeeding the extra-vascular space expands relative to the intra-vascular space. Transferrin has a greater distribution than albumin in the intra-vascular compartment. The effect of changes in these spaces on the serum concentration of these proteins is unknown, but it is reasonable to assume that they are of relevance. Whatever the factors and interaction, the net effect over the period studied was that the concentration of transferrin increased in both groups while that of albumin was unchanged, even though adequate dietary nitrogen was provided.

In severe iron deficiency anaemia there is a limitation in the use of transferrin as an index of protein nutritional status in that transferrin concentrations are increased (22), causing potential false negative errors. This did not apply in this study as the regimen for both parenteral nutrition and the formulae used for tube feeding both contained iron. Transferrin has no role in iron absorption even when fully saturated by the intravenous infusion of iron (23). Transport of iron may also be compromised at very low concentrations (18) but to try to prevent this there is almost certainly a feedback mechanism by which transferrin synthesis is stimulated by a falling iron so that transport is maintained. One other point should be made in the consideration of transferrin in the nutritional role. There is accumulating

evidence that some microbes have the ability to withdraw iron from transferrin by their ability to produce iron transport compounds. The greater the saturation of transferrin with iron, the better do these organisms flourish. Thus in patients with low transferrin, which is more highly saturated, there is more chance of a successful fungal or bacterial infection developing. This would have the effect of further reducing the transferrin concentration as the synthetic rate is lower in infected patients than the catabolic rate (143). Hence the supplementation of diets with iron may not be advantageous in infection, as this may compromise what seems to be a delicate defence mechanism.

In this study, in which sepsis and stressful events were eliminated as far as possible, transferrin was shown to be of more value than albumin in monitoring early nutritional repletion. Young and his colleagues have shown that transferrin concentrations could be maintained or restored in two weeks after surgery by intravenous hyperalimentation, giving 36.5 Kcalories/kg and 0.23 g nitrogen/kg per day, as long as sepsis did not occur (165). Their data on albumin showed that there was an almost uniform decrease in concentration but this was small and not outwith the normal limits.

Shetty and his colleagues have recently reported results which are in agreement with this finding in obese women given varying amounts of protein in their diets (144). As in others' findings in children they found retinol binding protein, then prealbumin and then transferrin preferable to albumin in monitoring dietary treatment.

ENERGY REQUIREMENTS FOR NITROGEN BALANCE

Nitrogen balance was better in the IVH group who had a significantly higher calorie intake than the ENT group. Even in the former the intake was greater than that planned as optimal, 1.88 x BEE as opposed to 1.76 x BEE. Although the apparent net protein utilisation and nitrogen balance were better than for enteral feeding, this latter was at a lower level of energy intake, and no differential improvement in the concentrations of albumin or transferrin were noted.

Increasing dietary energy intake improves protein utilisation both in the healthy volunteer with low oral protein intake (145) and in patients receiving intravenous hyperalimentation (146). Had equivalent amounts of calories been given both the predicted nitrogen balance and net protein utilisation would have been better for enteral feeding than for intravenous hyperalimentation. Thus using Figures 1 and 2, at the mean energy intake for enteral feeding of 1.53 x BEE the nitrogen balance would have been +2.4g with the NPU 60% whereas for IVH with this energy intake nitrogen equilibrium was only just obtained with NPU of 47%. The comparable predicted figures at the mean caloric intake for IVH would have been nitrogen balance of +2.4g with NPU of 58% for intravenous hyperalimentation and nitrogen balance of +5.0 g with NPU of 73% for enteral feeding. Thus enteral feeding is a more efficient method of utilising supplied nutrients. There is also the advantage that intake of nutrients via the portal circulation into the liver after normal enteral feeding favours hepatic protein synthesis rather than that in skeletal muscle (147).

There are a number of possible explantations for the relative inefficiency of intravenous hyperalimentation. Intravenous hyperalimentation differs physiologically from normal feeding in that there is a continuous infusion of nutrients as opposed to successive periods of absorption and fasting. Further, any infusion delivers nutrients into the systemic system which bypasses the normal route of entry and assimilation through the gut into the portal circulation and hence into the liver. Peripheral tissues, particularly skeletal muscle and adipose tissue, are presented with a constant supply of glucose and amino acids which, due to the anabolic stimulus from the resulting high insulin concentrations, are preferentially taken up (148). The relative importance of this finding is uncertain, but the amino acid profile in the circulating plasma may be modified. Thus the liver may compete unfavourably for certain of the circulating amino acids and protein synthesis, which is so dependent on amino acid supply, may be compromised. In contrast, Ellwyn has shown that the initial passage of a protein load through the liver leads to an increase in liver and plasma protein synthesis (149).

Nevertheless one effect of the continuous infusion of nutrients is the conversion of some of the infused glucose to glycogen and fat in the liver. This accounts for the ubiquitous finding of fatty infiltration in liver biopsies taken from patients on long term intravenous hyperalimentation together with the abnormalities in liver function tests. Energy is thus wasted in fat deposition (15). Another effect of these infusions is that the balance normally found between feeding, absorbing and fasting becomes

non-existant. There is therefore no opportunity for mobilisation of body fat as the constant high insulin stimulated by hypertonic dextrose (151), prevents the breakdown of stored fat. A useful energy reserve is thus unavailable, and also the failure to mobilise the fat stores may lead to deficiency in essential fatty acids (152, 153).

It may well be that the technique of "cyclic hyperalimentation" as described by Maini et al will be shown, as initial reports have strongly suggested, to overcome many of the drawbacks, yet without loss of the benefits of intravenous hyperalimentation (154, 155, 156). By this technique the infusion of hypertonic dextrose is discontinued for a period of eight to 12 hours each day, thus allowing a post-absorptive state to develop. Fat and glycogen deposition in the liver is less, liver function tests can return to normal, nitrogen retention is comparable (154) and essential fatty acid deficiency may be prevented (156).

A further explanation of the relative inefficiency of intravenous hyperalimentation may lie in the relationship of carbohydrate intake and thyroid metabolism. Danforth and his colleagues in a preliminary study (157) reported that the serum concentration of triiodothyronine and hence the metabolic rate is directly related to the dietary carbohydrate concentration. With the greater amount of carbohydrate provided in IVH it can be argued that greater heat production would follow from increased thyroid function and thus there would be a possible wastage of the calorie intake. Against that view can be forwarded the suggestion that if the metabolic rate is increased, much of the energy expended

would be used for useful work such as transport of various substrates and ions and to increase the rate of some processes through greater heat production.

In this study the extra calories provided during IVH did produce a better nitrogen balance, but without any improvement in hepatic secretory function as assessed by the serum concentrations of albumin and transferrin. The rise in transferrin concentrations was similar in both groups, but not more so in the IVH group. Assuming that nitrogen was retained, it is most likely that this was in skeletal muscle (158). Two quite separate mechanisms may account for this. In subjects fed protein meals, there are increased amino acid levels found after eating, of which the branched chain amino acids, valine, leucine and isoleucine, are the major contributors (159). These particular amino acids are not taken up readily by the liver, but play an important part in muscle nitrogen repletion by their action in stimulating muscle protein synthesis (160). The other mechanism relates to the high insulin levels when glucose and amino acids are supplied. Hyperinsulinaemia appears to stimulate the uptake of the branched chain amino acids by muscle (161), thus increasing their intracellular availability for protein synthesis. With IVH therefore in which the glucose and insulin levels are much higher than with enteral nutrition, more amino acid will be retained in muscle.

It is therefore concluded that because of the probable increased uptake of dietary protein in muscle, loss of energy in heat production and conversion of infused glucose to fat, intravenous hyperalimentation is less efficient as a means of restoring the

levels of plasma proteins secreted by the liver, than enteral nutrition.

THE EFFECT OF PROTEIN SPARING ON THE METABOLIC RESPONSE TO SURGERY

Surgery leads to profound changes in the mechanisms by which an individual obtains and uses energy. These changes have been described in the introduction to this thesis. In the study of low dietary energy infusions after surgery two main questions about the patterns of the various energy fuels are raised. The first is whether the results which were obtained from the patients who were given either isotonic amino acid solutions or dextrose after surgery are comparable with other similar studies, both in the terms of the substrate response and the nitrogen balance. The second and more interesting question is whether by altering the metabolic environment to that of partial ketosis prior to surgery, the response to surgery was altered and, if so, whether this was beneficial, deleterious or of no consequence.

In broad outline the concentrations of each of the energy fuel substrates in Group B, given post-operative amino acids, and in Group C, given 5% dextrose, together with those of insulin after surgery are in accord with those found by other investigators (54, 113). For each group the mean concentration of each of the fuels on the morning before surgery is similar to that expected after an overnight fast. The effect of surgery was to initiate the mobilisation of further energy sources, as has been discussed earlier, with rises in the plasma concentrations of glucose, lactate, free fatty acids and ketone bodies. In the group given amino acids there was the expected pattern indicating a transition

to fat as the main energy source once the early response to surgery had passed, whereas a different pattern was observed in the dextrose group, primarily due to the supply of the small amount of carbohydrate.

Differences were noted in the substrate profile in the group who were given a carbohydrate-free protein containing diet before surgery, compared with the other two groups. These were particularly evident priot to surgery and in the profile from blood taken immediately after each operation had finished, but they were readily explained on the basis of the pre-operative adaptation. They will be discussed in greater detail in subsequent paragraphs.

The response in Group C, the group given dextrose in hypocaloric quantities following surgery, were as expected. The blood glucose concentration rose markedly after surgery due to the mobilisation of hepatic stores of glycogen and other gluconeogenic processes described earlier. This was found in association with a three fold increase in insulin. The high free fatty acid concentration was also expected due to the lipolysis which occurs during the early reaction to stress, through the action of the catecholamines and glucagon. The rise in lactate levels are comparable to those reported by Foster et al (111), and may be attributed to a number of factors. Glucose oxidation is inhibited with increased Cori cycle activity stimulated by the catecholamines (162). Tissue anoxia and acidaemia which occur in the early post-operative period will favour the shift towards lactate in the dynamic equation,

$$\text{Lactate} + \text{NAD} = \text{Pyruvate} + \text{NADH}_2$$

Further, the supply of glucose over the period of surgery would inevitably lead to the catabolism of some of the glucose and hence the concentrations of its metabolites, of which lactate is one, are raised.

The slight rise in total ketone body concentration after surgery is not in proportion to that of free fatty acids. Nevertheless this indicated fatty acid degradation in the liver in the face of a raised insulin, albeit with resistance to the effect of insulin already present.

As the immediate stress of surgery passed, the pattern of a falling blood glucose was evidence, but this varied little between the second and sixth/seventh days. The fall was due to the improved glucose utilisation which is found in the flow phase as insulin resistance lessens. Continuous glucose infusions maintain the concentration but only at a level slightly higher than the pre-operative fasting level. A further indication of the resolution of the metabolic response to surgery was the falling free fatty acid, lactate and ketone body concentrations to those found at fasting. This was achieved in the presence of an insulin concentration which remained very considerably above the fasting level, almost certainly due to the constant stimulus for insulin production which follows glucose infusions. It was of interest that the highest insulin levels were found not immediately after surgery, but on day 2 at which time there was already a decline in the glucose, free fatty acid and ketone body levels. This would indicate that there was still a considerable tissue resistance to insulin, and for its action to be effective, high concen-

trations were required. The return of the free fatty acid and ketone body concentration to those levels found prior to surgery illustrate the comment which has been made earlier, that suppression of energy release from fat stores may be deleterious in that energy is required to be provided from elsewhere, and this has to be protein stores.

The metabolic profile in the patients in Group B who were given isotonic amino acid infusions after surgery, is quite distinct from the group just described. The initial concentration of all the body fuels and insulin were similar in both groups and this similarity was also evident after surgery, whether electrolyte solutions or dextrose had been infused during the operation, illustrating the common response which occurs whatever the energy supplied. However once the ebb or acute phase had merged into the flow or adaptive phase, the profiles showed differing patterns. That in Group B demonstrated the pattern of developing keto-adapatation. With no carbohydrate intake the blood glucose concentration had dropped by day 2 to a level somewhat lowere than that found after an overnight fast. This level was maintained over the next four to five days, indicating that some glucose was being synthesised, either directly from the glucogenic amino acids or through the activity of the Cori cycle.

It is the development of the metabolic environment of ketoadaptation which is analagous to that found in fasting with fat mobilisation and raised free fatty acids and ketone bodies which has been emphasised as the crucial factor for protein sparing therapy to be effective. In Group B this developing pattern was evident

with free fatty acid levels falling after the high peak which followed surgery by day 4, but rising by day 6/7. The ketone body level showed a steady rise, the insulin concentrations dropped to fasting levels. These changes compare with previously reported studies (54, 65, 67, 115). However a major problem for some workers in this field has been their inability to reproduce the high ketone body concentrations reported in this and other studies (66, 111). Their profiles have tended to compare with patients infused with dextrose and because of this they challenge the proposed mechanism of protein sparing as due to low insulin levels.

Close examination of these latter reports is necessary because certain features become evident. Amino acids have a varying stimulus on insulin production. In Craig's study (66) a very much higher amount of amino acid was infused than in other studies, sufficient to give 22 grams of nitrogen per day. In all probability this would produce the additional stimulus for insulin secretion which would inhibit fat mobilisation and hence reduce the keton body level, as indeed was observed. The time over which any study is carried out is also of relevance. Depending on the surgical stress, three or four days may be insufficient for adaptation to be evident. A further criticism which could fairly be directed at Foster's study is that broad conclusions should not be drawn from results from a single patient (111)!

There were very considerable differences in the pre-operative profile in Group A patients, those given a carbohydrate free diet for two days before surgery, compared with the other two groups.

The glucose, free fatty acid and ketone body concentrations confirmed that the planned adapatation to a ketotic state had occurred. It is not argued that this adaptation was complete as some three to four weeks are necessary for full adaptation. Nevertheless after only two days there was a shift towards the use of fat as an energy source. The glucose concentration was lower than that in the other two groups, due to the loss of carbohydrate stores of glycogen in the liver over the preceeding two days. The failure to respond to surgery with a similar rise in glucose as in the two other groups is further evidence that there was a shortage of readily available carbohydrate. The level was raised, the likely source being glucogenic amino acids. It is of interest that the free fatty acid level hardly changed in this group after surgery whereas in the other groups there was a rise to comparable levels. This rather indicates that lipolysis was already well established. There was a slight fall by day 2, but further rise. The ketone body concentration dropped slightly after surgery and remained much the same on day 2, suggesting that utilisation may have been greater. It would seem from these results that fat mobilisation was already at an optimal rate in contrast to Groups B and C. Whether there was increased lipolysis over the period of surgery and for a couple of days thereafter cannot be stated as the figures reported only quantitate the serum concentration, and do not bear any relationship to rate of synthesis or utilisation.

The insulin concentration in this group was slightly higher than in the other two groups before surgery, but with the ketoadapted state, albeit partial, the level could be expected to be lower

than after an overnight fast. Statistically there was no significance in the levels, yet it is possible that despite the low glucose concentration, the raised fatty acid and ketone bodies were exerted a direct stimulatory effect (62). The post-operative decline in insulin concentrations was steady, dropping by day 6/7 to 7 μ units/ml which is of the same order as that previously reported after a similar period of protein sparing therapy in less stressed individuals (113).

In summary, the data from these various assays is consistent with the intended aim of manipulating the metabolic environment in Group A patients to a partially ketoadapted state. The statistical technique employed confirmed that the two groups receiving amino acids differed from the group receiving glucose in their response not to surgery, but the available energy source after the initial surgical stress had passed.

Only with ketone body results was any difference between Groups A and B noted. This would suggest that there was no particular value in pre-operative ketoadaptation, yet trends could be seen in that the ketone body concentrations remained higher in Group A than in Group B and the insulin concentrations fell sooner to lower values. It could be argued that in Group A the acute phase was shorter but whether this was an effect of pre-operative ketoadaptation or a reflection of less overall stress in the patients in this group is open to debate.

From the above discussion it is evident that those patients who were partially ketoadapted prior to surgery, attained the similar

ketotic state sooner than the group in whom amino acids were infused only after surgery, although by the end of a week there was little difference in the profiles between the groups. Whether any benefit was obtained by manipulating the pre-operative environment would only be identified by an improved nitrogen balance or, as the aim of protein sparing therapy is the preservation of nitrogen, the lesser decline or more rapid restoration of any protein "marker". This latter aspect will be discussed later.

The nitrogen balance data is of interest. In Group C in whom there was no nitrogen intake, the nitrogen balance is essentially a measure of the loss of nitrogen over the period under study. The loss abated somewhat from the early days after each operation, stabilising to around 8.4 - 9 g/day. This loss is similar to that reported by Freeman's group (113) of a mean daily loss of 9.7 g. The administration of amino acids improved the nitrogen balance, that is to say that although the gross loss was certainly greater, the net loss, once that nitrogen supplied had been corrected for, was less. In both Groups A and B this was evident, but with the balance in Group B better. Closer examination of these two groups reveals certain apparent inconsistanceis. In Group B there was a much better mean nitrogen balance over the first four days (-1.4 to -3.8 g with a fairly wide standard deviation) than over days 5 to 7 when the mean was fairly stable (around -5.0 to -5.5 g/day). With the acute phase of the metabolic response to surgery and the resulting protein catabolism, one would have expected a greater loss in the early days, whether the urea excreted was derived from muscle protein or from the infused amino acids.

However, it may be, as Freeman has pointed out (115), that with an intake of dietary nitrogen, several days are required before the increase is reflected in urinary nitrogen excretion.

A more likely explanation is that by the end of the week the actual intake of nitrogen was less as patients got better and were allowed oral fluids in small amounts. This allowance often resulted in a slowing of the infusion rate. The reduction of infusate by 500 mls decreases the nitrogen intake by some 2 grams depending on the particular amino acid solution used. There was improved nitrogen balance in Group B over Group A, though not of statistical significance. The mean daily intake in Group B was 1.2 g nitrogen higher than in Group A. Freeman has shown that by improving the "protein" intake from 1.0 g/Kg to 1.7 g/Kg the nitrogen balance is also improved, but without affecting the free fatty acid, and ketone body levels both in normal volunteers and in patients after surgery. In the latter the nitrogen balance at 1.0 g/Kg/day was -8.9 g/day which is somewhat comparable to the results obtained by Group A whose intake was also 1.0 g/Kg/day (113, 115).

Thus in terms of nitrogen balance, no improvement was seen by partially ketoadapting patients prior to surgery.

In this study only the group data of nitrogen intake and balance has been presented. The actual nitrogen losses in patients receiving amino acids was considerable, a point which others have made (115). This implies that a proportion of the amino acids were not assimilated, but whether the increased loss represents

direct excretion of infused amino acids or metabolically derived nitrogen containing products such as urea from amino acid metabolism was not tested in this study. Certainly there was a considerable increase in the urinary urea excretion as measured. In this study a fixed increment of 2 grams per day was added to the urinary urea nitrogen to account for skin, intestinal and non-urea nitrogen losses in the urine. In this respect there may be differences from others' method of measuring nitrogen loss (65, 67, 115), but is in accord with the practice of the Laboratory from which these results came (112).

The mechanism of protein sparing remains uncertain. The nitrogen sparing effects of a carbohydrate free protein or amino acid "diet" have been attributed to the mobilisation and utilisation of body fat in the presence of a low circulating insulin concentration. The addition of glucose to amino acids is undoubtedly a stimulus for insulin secretion which would inhibit fat mobilisation and lead to less protein being spared and hence to a poorer nitrogen balance, as demonstrated by Blackburn's group (54). However, the nitrogen balance has not been found to be any different with the addition of glucose by either Greenberg (65) or Freeman (115) and their teams. They therefore concluded that nitrogen sparing was not totally due to lipolysis and that the importance of a low circulating insulin had been overemphasised.

This conclusion, when used to criticise adversely the mechanisms proposed by Flatt and Blackburn (51), tends to ignore the very flexible nature of the metabolic fuel cycle with its variable leverage exerted by differing metabolic fuels.

An alternative explanation for the nitrogen sparing action of amino acid infusions has been that the quantity of amino acids is itself the crucial factor. Hoover has suggested that the endogenous amino acids are spared catabolism because a proportion of the amino acids utilised are those infused into the amino acid pool, although precisely how much body protein is thus spared is uncertain (67). Freeman's work in which enhanced nitrogen balance was found with increased amino acid infusions is in broad agreement (115).

Although this may account for some of the protein sparing effect, there are other possible explanations which are less obvious. The infused amino acids enter amino acid pools in muscle, liver, in the circulation and in other organs. In muscle they shift the metabolic equilibrium in the direction of reduced amino acid release from the tissue, and in the liver towards increasing metabolism of carbon skeletons and production of urea from amino acids (111).The branched chain amino acids, which are raised in fasting, are known to promcte a more positive nitrogen balance through the operation of the branched chain amino acid-alanine cycle in which the increased oxidation of branched chain amino acids during fasting could provide energy for muscle. The resultant synthesis of alanine from glucose would be used in the transfer of ammonia and glucogenic percursors back to the liver (163). Branched chain amino acid concentrations are raised also in amino acid infusions, but not when amino acid and glucose combinations or glucose alone are infused (65).

This observation may be crucial to the understanding of protein

sparing. It has been emphasised earlier that the measure of nitrogen balance only indicates an overall gain or loss of nitrogen. If there is a gain, and this gain is primarily by muscle, which is certainly compatible with the action of insulin stimulated by a glucose/amino acid infusion, with a probable inhibition or at least decrease of transfer of amino acids from muscle to other visceral organs, then there may be a shortage, albeit relative, of amino acid supply to these various other organs and hence in those pathways where rate limiting steps may be related to the supply of amino acids. As protein supply may be a factor in the rate of synthesis, the rate of production may be slowed. Thus, despite the assertion that a low insulin concentration may have been overemphasised, it can be argued cogently that with a low insulin concentration the metabolic environment, which is similar to that found in fasting, does not hinder the potentially useful transfer of amino acids from muscle to liver, whereas if glucose is added, there may indeed be an equivalent uptake of amino acids, but they are less usefully employed in building up muscle protein stores. The role of the branched chain amino acids certainly requires further study, and indeed it will be of great interest to note whether the infusion of branched chain amino acids alone produced equivalent nitrogen balances to that achieved with the currently available amino acid solutions.

THE EFFECT OF PROTEIN SPARING THERAPY ON NUTRITIONAL MARKERS

Two main questions require to be answered in discussion of the potential role of protein sparing therapy as supplying the

visceral protein as opposed to the skeletal muscular component of the body cell mass. One is whether the improved nitrogen balance found with amino acid solutions is associated with improved concentrations of protein which correlate well with nutritional states and which are secreted in the viscera, and particularly by the liver. The main argument is that of the amino acids supplied, a proportion is used for the synthesis of glucose and hence energy production, thus "sparing" the degradation of muscle or other protein for this purpose. This occurs in fasting and once the acute phase of the stress response has passed, and will continue to some extent once full adaptation to the use of ketone bodies as an energy source has taken place. The rest of the amino acids increase the pool in the plasma and are taken up by the liver rather than by muscle and therefore they provide the necessary continuing stimulus and substrate for hepatic protein synthesis.

The concentration of protein such as those designated as nutritional "marker" proteins would be expected to be maintained in a situation of minimal stress, but after surgery there should be less of a fall or alternatively a more rapid restoration than would be achieved by any intravenous regimen in which no amino acids were made available, nor was there any opportunity for fasting ketosis to develop. Clearly the concentrations would depend on many factors which have been discussed earlier, but if the half-life of the protein is related to its synthetic rate, then prealbumin of the proteins assessed here should be restored reasonably early. Although it is known that the synthetic rate of albumin is enhanced by amino acid infusions (97), it is not known whether the same

holds true for transferrin or for prealbumin. For albumin the synthetic rate is 180 - 300 mg/Kg/day (6), for transferrin it is 6 mg/Kg/day (164) and for prealbumin it is 2 mg/Kg/day (19). Catabolic rates will alter after surgery or with infection. Further redistribution of the proteins within the tissue spaces particularly into the oedema filled tissues around the operative site will also be of relevance.

In all groups there was no significant fall in the concentration of any of the three proteins in any group during the actual operation, indeed a small rise was recorded for some of the proteins, in Group A and Group B for albumin and Group B for prealbumin. These rises were of no statistical significance. A possible explanation is that salt poor albumin or purified protein fraction were infused during surgery to a certain number in each group, together with blood as needed. Even so as there was no overall fall, it is suggested that whatever mechanisms exist, whether in the redistribution throughout tissues, catabolism or reduction of synthesis, these were only intiated during the time of surgery.

The patients in Group A who were given pre-operative protein sparing therapy were shown to maintain their marker protein concentrations better than those given conventional post-operative protein sparing infusions or only glucose, whereas there was no difference in this respect over the period of the study between the latter two groups. It is tantalising to speculate on what the further pattern of the proteins would have been had the study been prolonged, but this could not be justified as the patients

were ready to return to more normal nutrition. It is probable that the real benefit of protein sparing therapy over dextrose is obtained from this time onwards when the stress of surgery has passed, as indeed has been suggested by Bistrian and his colleagues (3) who observed a continuing fall in albumin concentrations in patients receiving dextrose for longer periods. Their patients receiving amino acids did demonstrate reasonable albumin concentrations. In this study the numbers were too small to subdivide the groups into those nourished or malnourished, those infected or non-infected or to present useful data from any given individual.

There has, subsequent to this work being completed, been a report from Young and Hill of a somewhat comparable study in which the nutritional "marker" proteins, albumin, transferrin, prealbumin and retinol binding protein together with many of the acute phase proteins assayed in this study, were evaluated with differing post-operative intravenous regimens (165). The surgical procedure, proctocolectomy or abdomino - perineal excision of the rectum, could be considered of comparable stress to that undergone by many patients in this series. One of their groups of patients received no nutritional supplements, the second received amino acids in a slightly more concentrated solution than used in this study (4.25% as opposed to 3.5%) and the third group were given full intravenous hyperalimentation, with 36.5 Kcal/kg/day. Their results are of interest in so far as they relate to the protein concentrations, but their comment "After surgery all patients were allowed an unrestricted food intake" should be noted, particularly when putting the results of 15 days following surgery into context.

At this time after surgery the mean concentrations of transferrin, prealbumin, and retinol-binding protein were significantly lower than normal for the control (first) group and for the patients receiving only amino acids, whereas they were normal for those who received full intravenous hyperalimentation. It is of interest that in all groups the albumin concentrations remained within normal limits by 15 days following surgery.

When the paired data from each patient were analysed, there was a consistant decrease in the control group, but in those receiving amino acids there were inconsistant changes, but generally there was a fall after surgery. The presence of sepsis was noted to cause a more profound drop in the concentration of each protein, which took longer to be restored. Nevertheless, with the data selected to eliminate all septic patients, a similar patter to that of the groups as a whole was demonstrated.

From their study, Young and Hill were unable to substantiate that enhancement of the synthesis of these marker proteins as reflected by the plasma concentration took place with the infusion of amino acids. Their findings therefore did not corroborate those of Blackburn and Bistrian (76), but they are in broad agreement with the results obtained from Groups B and C in this study in which no clear cut benefit was found by the infusion of amino acids in the early post-operative period using these marker proteins as indices.

Another comment is worth making about the report from Young and Hill (165). By giving intravenous hyperalimentation the nutritional

status was shown to be restored by two weeks after surgery, at least as assessed by the marker proteins. It is not appropriate to compare this method of feeding with what is certainly a hypocaloric regimen except to note the obvious benefit. Whether it can then be argued that the benefit sufficiently outweighs the cost to use this method of nutrition as a routine after certain surgical procedures involves the discussion of cost, risks and other factors.

There is however a second question to be answered. That is whether the metabolic adaptation of inducing a partial ketotic state prior to surgery led to a differing or improved "marker" protein profile. From the results of this study the conclusion is that there was a difference as each of albumin, transferrin and prealbumin, the latter to a lesser extent, showed a much reduced concentration, when compared to the group who only were protein spared after surgery. The nitrogen intake was similar, even though a little less in the former group. Yet despite this intake in Group B, and the biochemical adaptation to ketosis after surgery, and the improved nitrogen balance, there was no improvement in the protein profile over Group C, receiving dextrose, whereas Group A was different. Two possible mechanisms are suggested, which may be of relevance.

Munro (166) has described the loss of labile body protein which occurs with a protein-free diet. Labile protein stores can be considered as available protein which provides stability throughout the variety of nutritional conditions to which an individual is subject, and thus there may be rapid changes in the protein

content of tissues such as the pancreas, gut mucosa and liver in response to changes in protein intake. The "buffering" effect of this protein may also explain why the catabolic rate of albumin does not decrease immediately after protein deprivation (8). It is reasonable to postulate that with the low calorie intake in protein sparing therapy, some loss of labile protein will occur, perhaps also made available as glucogenic precursors or to continue to supply essential substrate for protein synthesis. It is also known that previous dietary intake has a bearing on the catabolic response to injury (166). In subjects with a high level of dietary protein intake the response to injury is more severe. Thus it can be postulated that with the reduced pre-operative food intake in Group A patients, with less labile protein, the catabolic loss of protein as substrate for acute phase and other protein synthesis was less than in the other groups.

Perhaps a more likely explanation for the differing response in Group A is the effect of ketone bodies on muscle metabolism. Sherwin and his colleagues have shown that their infusion during fasting conserves nitrogen even more than that due to the adaptation to fasting (167). This may be due to the reduction of branched chain amino acid oxidation which in turn leads to an overall decrease in muscle protein catabolism (160). As the ketone body concentration in Group A dropped only slightly from the raised pre-operative level for a couple of days, and then rose to higher levels (Table 7), it is reasonable to conclude that there was a much greater reduction of muscle degradation in this group than the others, that ketones were available in

reasonable quantities to supply energy, and that therefore much of the infused amino acids could be used for anabolic processes.

It has already been mentioned that the branched chain amino acids may have a stimulatory effect on albumin synthesis in the liver (13, 14) and that branched chain amino acid levels are raised with amino acid infusions (65). It is therefore possible that another reason for the level of albumin being held may be the direct stimulatory action of the branched chain amino acids.

THE EFFECT OF PROTEIN SPARING THERAPY ON THE ACUTE PHASE PROTEINS

The data presented for the concentrations of the various acute phase reactant proteins prior to surgery indicates that manipulation of the metabolic environment to a ketotic state has no influence on the unstressed levels of these proteins. In all groups there were very similar concentrations. It is however perhaps worth commenting on the level of C reactive protein as in each group the mean was above the upper limit of normal quoted by Cleve (168). One patient only in each group started off with a raised level of C reactive protein within the range of 12 - 15 mg/l. This had the effect of raising the group mean somewhat artificially. C reactive protein can be used to monitor inflammatory stress and although one of the patients, in Group A, had a mild ear infection which may have been relevant, no obvious cause was found in the other patients. Malignant disease has also been reported as a cause for elevated concentrations of the acute phase proteins (168), but despite the numbers in each group with malignancy, the baseline levels of the other proteins were not raised.

The effect of surgery was to initiate the acute phase response. In this study the timing of such increases in the protein levels, and the pattern of their rise, at least in Groups B and C, were comparable to other reported series (92, 93). The pattern as established by Fischer and his team is that C reactive protein is the first to rise, within six hours of injury and peaking at two days (169). This is followed by α_1-antitrypsin, α_1-acid glycoprotein, C_3 complement and haptoglobin, all of which tend to peak around three to four days. Caeroluplasmin demonstrates a much later rise, usually after a week and indeed in this study no appreciable change had been noted by the end of the first week.

In precise terms the changes in Groups B and C were less than those in other series, both in absolute concentrations or as a percentage above the baseline level (92, 93). This supports Werner's contention that the severity of surgical stress is unrelated to the size of the increase (92). However if inflammatory stress is prolonged the return to normal may be delayed.

In that there was no difference in the pattern between groups of patients infused with dextrose or with amino acids, no argument could be made for suggesting that supplying protein was of value. Indeed it is of considerable doubt that the degree of response as measured by the concentration in the serum bears any relationship to nutritional status or support. The high priority afforded to the synthesis of these proteins is probably such that whatever the adaptation to the available energy fuels, sufficient amino acid will get to the liver to act as substrate for acute phase protein synthesis. Dietary supply is clearly not the limiting factor.

This is not to say that the serial assays of one or more of these proteins would not be useful in monitoring the course of a given patient after surgery or with sepsis. Fischer et al have suggested that the assay of C reactive protein would be sufficient on its own for such a purpose (169).

Nevertheless there was a slight difference noted for the group ketoadapted prior to surgery in that there was less of an increase with a more gradual rise to the peak of those proteins which did demonstate the expected increase. The early post-operative concentrations of α_1AG, α_1AT, haptoglobin and C_2 fell in Groups B and C, whereas this was only so to a minimal extent in Group A or else there was a slight increase. The relevance of this observation is uncertain as it suggests either that Group A patients were a different population, which by the attempted randomisation they should not have been, or that in some way the metabolic adaptation did in fact modify the acute phase protein response. That the relative inhibition of muscle catabolism from the high ketone body concentration might lead to a reduction in the supply of a necessary amino acid needed in the synthetic pathway is difficult to accept as the solutions infused contained both essential and non-essential amino acids. The most obvious explanation is that there was less "infective" or other stimulus for these proteins to be synthesised. There was only one patient who was recorded as being infected in this group, but this does not account for the observation from others that the pattern of increase of these proteins appears to be constant merely as a result of the surgical procedure. I cannot account for this observation, but must assume that a metabolic modification had

taken place.

The lack of correlation between ketone bodies and the acute phase protein response either individually or as groups is not unexpected from what is known of the stimulus for secretion of the proteins. Deep surgical sepsis leads to loss of ketonaemia through increased insulin secretion (170), or inhibition of free fatty acid oxidation (171) with worsening nitrogen balance. Minor infection does not appear to cause this extra loss of nitrogen when amino acids were infused (172). Infection is associated with release of pyrogen and leucocyte endogenous mediator, proposed triggers for acute phase protein synthesis. Statistical analysis to see if there was an inverse correlation between the ketone body concentrations and the acute phase proteins was not carried out.

THE EFFECT OF PROTEIN SPARING THERAPY ON IMMUNOGLOBULINS

Two slightly differing reports have been published on the response of immunoglobulins to surgery or trauma. Ballantyne and Fleck (173) reported no change in IgA and IgG concentrations and a rise, but within normal limits, of IgM in the first ten days in two groups of patients with a variety of fractures. Aronsen and his colleagues noted a similar pattern for IgM, a similar failure of IgG concentrations to rise, but found that IgA also rose slowly a week after abdominal surgery using cholecystectomy as a standard operation (93).

In this study the immunoglobulins remained within the wide limits of normal concentrations, but nevertheless, significant changes were found within these limits by statistical analysis for the

effect of time. The response of the immunoglobulins to major surgery, perhaps because of the nature of opened tissues with the possibility of infection and earlier stimulation of the immune system may differ from that found after closed fractures. It is likely that further changes would have been evident had the period over which the study was carried out been extended.

It is probably inappropriate to comment on the patterns observed for each of the nutritional regimens except to indicate that where there was a post-operative fall in concentrations and then rise up to or above pre-operative baselines, there was less movement either way for Group A, a similar trend to that observed with some of the acute phase proteins. The clinical significance of this observation is unknown particularly when there is no clear cut evidence that amino acid supply is of benefit to synthesis of the immunoglobulins, although this is most probable, nor that there is any correlation between immunoglobulin concentration and nutritional status. One certain conclusion which is valid is that the serial measurements of the immunoglobulins are unhelpful in providing or disproving the theory that protein synthesis in the liver is enhanced by amino acid infusions after surgery. In this study the observation that in Groups A and B, who received amino acids, there were fewer days of fever and fewer infections supports the general principle that nutritional support is of benefit, but the parameters chosen for the effect on immune function did not demonstrate this.

CONCLUSION

In the study on indices of protein repletion in adult patients, transferrin was shown to be a valuable index of nutritional repletion in the unstressed patient.

Energy requirements sufficient to produce nitrogen equilibrium with an adequate nitrogen intake were calculated. Enteral feeding gave better nitrogen balances for any given dietary intake than did intravenous hyperalimentation. For the latter route the energy requirement of 42 Kcal/kg to produce nitrogen equilibrium is comparable to that reported elsewhere (116, 136). The greater efficiency of enteral nutrition has been ascribed to the more normal uptake of nutrients through the portal system to the liver, with its variable pattern of eating, assimilation and fasting in contrast to the wasteful use of energy leading to fat deposition found with continuous infusions of hypertonic glucose solutions, possible greater loss in heat production through dietary stimulation of the thyroid and also greater uptake of nutrients into muscle due to the prevailing increased insulin concentration.

After major abdominal surgery neither the acute phase proteins nor the immunoglobulins were found to be of value as nutritional markers, nor was there any clear difference observed over the first week following surgery in groups given amino acids or glucose infusions. However, if patients were rendered partially ketotic prior to surgery, a different pattern was noted. A similar difference was noted for the profiles of albumin, trans-

ferrin and prealbumin in which the pre-operative concentrations were better maintained. It is suggested that by this metabolic manipulation the time in which adaptation to fat as a main energy source occurs after surgery is reduced and that the benefit is due to the higher concentration of ketone bodies which reduce skeletal muscle catabolism and increase the availability of infused amino acids for protein synthesis in the liver.

In this light the observation by O'Keefe et al (174) that after surgical injury protein synthesis is improved when amino acids are infused, without any change in protein catabolism, emphasises that an anabolic response to stress does exist if suitable substrate is provided. This finding challenges long-held views in which the response to surgery has been considered to be solely catabolic in nature. If substrate is not provided net protein catabolism does occur and the anabolic processes are masked.

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TABLES

FIGURES

NOTE TO FIGURES 5-22

In each of Figures 5-22, which are copies of a computer print out, the data shown for "Day-1" is that appropriate for each group as the mean pre-operative concentration on the morning of the day of surgery. The data shown for "Day 0" is the mean post-operative concentration taken within two hours of the end of the operation.

TABLES

TABLE 1 - STUDY I - CLINICAL CHARACTERISTICS

STUDY 1

Patient	Sex	Age	Diagnosis	Days	Initial Albumin g/dl	Final Albumin g/dl	Change g/dl	Initial Trans-ferrin mg/dl	Final Trans-ferrin mg/dl	Change mg/dl
IVH GROUP:										
J.W.	M	65	Zollinger Ellison Syndrome	12	3.4	3.8	+0.4	180	220	+40
G.S.	F	66	Oesophageal Carcinoma	15	4.1	3.9	-0.2	92	160	+68
E.H.	F	63	Malabsorption/Jejunoileitis	9	2.9	3.5	+0.6	100	238	+138
R.B.	M	56	Ca Bladder-Small bowel Fistula	13	3.1	3.2	+0.1	80	155	+75
M.S.	F	21	Anorexia Nervosa	12	4.4	3.7	-0.7	184	280	+96
C.L.	M	50	Stricture Common Bile Duct	12	2.4	2.6	+0.2	140	160	+20
J.C.	M	66	Short Gut Syndrome	8	3.5	4.1	+0.6	184	220	+36
J.F.	F	51	Ca Bladder-Small Bowel Fistula	16	3.1	3.2	+0.1	165	180	+15
W.M.	M	61	Pancreatic Pseudocyst	11	3.9	3.3	-0.6	128	187	+59
J.C.	M	66	Short Gut Syndrome	8	4.3	3.2	-0.9	128	260	+132
ENT GROUP:										
B.G.	F	79	Arteriosclerosis	13	3.3	3.0	-0.3	184	172	-12
G.C.	M	51	Ca Pancreas	14	3.1	3.5	+0.4	160	234	+74
M.McD.	F	80	Coronary Arteriosclerosis - Post Bypass Graft	19	3.3	2.9	-0.4	156	220	+64
V.B.	F	71	Chronic Obstructive Pulmonary Disease	18	3.2	3.6	+0.4	234	350	+116
A.T.	F	66	Ca Lung	14	3.3	3.4	+0.1	172	196	+24
G.T.	M	69	Ca Lung - Post Lobectomy	20	3.0	3.4	+0.4	88	142	+54
G.A.	F	54	Ulcerative Colitis	12	3.4	3.5	+0.1	109	192	+81
A.B.	M	65	Lymphoma of Stomach - Post Gastrectomy	12	2.9	2.5	-0.4	165	193	+28
C.T.	M	81	Ca Pancreas - Post Biliary Bypass	14	2.5	3.1	+0.6	96	178	+82
G.C.	M	34	Ulcerative Colitis - Post Procto-Colectomy	17	3.0	3.0	0	156	280	+124

TABLE 2 - STUDY I - SERUM CONCENTRATIONS OF ALBUMIN AND TRANSFERRIN

STUDY 1

TABLE 2 - SERUM CONCENTRATIONS OF ALBUMIN AND TRANSFERRIN

	ALBUMIN (g/dl)			TRANSFERRIN (mg/dl)		
Group	Initial	Final	Change	Initial	Final	Change
ENT	3.1 ± 0.3	3.2 ± 0.3	+0.1	152 ± 44	216 ± 60	+64 ± 42*
IVH	3.5 ± 0.6	3.5 ± 0.4	0	138 ± 39	206 ± 44	+68 ± 43*

*$p<0.001$ by Student's paired t-test

TABLE 3 - STUDY II - CLINICAL CHARACTERISTICS

TABLE 3 - CLINICAL CHARACTERISTICS - STUDY 2

Group	Patient	Age	Sex	Diagnosis	Operation
A	A.B.	61	M	Carcinoma of bladder	Ileal loop diversion
	R.B.	16	M	Morbid obesity	Gastric bypass
	M.C.	75	M	Carcinoma of bladder	Radical cystectomy
	L.C.	69	M	Carcinoma of bladder	Radical cystectomy
	M.G.	57	F	Carcinoma of common bile duct	Laparotomy-Colostomy
	R.McD.	44	M	Chronic pancreatitis	Exploration of common bile duct, pancreatico-jejunostomy and gastro-enterostomy
	H.M.	71	M	Carcinoma of colon	Left hemicolectomy
B	T.C.	81	M	Carcinoma of bladder	Ileal loop diversion
	C.E.	64	M	Carcinoma of common bile duct	Choledochochole-dochostomy
	R.F.	63	F	Carcinoma of colon	Transverse and sigmoid colectomy
	T.F.	65	F	Carcinoma of bladder	Ileal loop diversion Radical cystectomy
	A.G.	67	M	Leiomyosarcoma of rectum	Pelvic exenteration
	H.G.	64	M	Carcinoma of bladder	Radical cystectomy
	C.La.	52	M	Stricture common bile duct	Choledochojejunostomy Posterior gastro-enterostomy
	C.Li.	42	F	Crohn's disease	Small bowel resection
	C.McD.	68	F	Carcinoma of bladder	Ileal loop diversion

(Over)

TABLE 3 Continued

Group	Patient	Age	Sex	Diagnosis	Operation
C	R.B.	55	M	Carcinoma of bladder	Ileal loop diversion
	R.G.	69	M	Carcinoma of bladder	Ileo-caecal loop diversion
	J.G.	84	M	Carcinoma of colon	Right hemicolectomy
	G.H.	43	M	Cholelithiasis, chronic cholecystitis	Cholecystectomy
	G.O'B.	50	M	Carcinoma of rectum	Anterior resection
	R.R.	30	M	Crohn's disease	Resection terminal ileum and caecum
	D.R.	81	M	Carcinoma of stomach (cardia)	Oesophagogastrectomy and splenectomy
	M.S.	60	M	Carcinoma of bladder	Ileal loop diversion Radical cystectomy

TABLE 4 - STUDY II - NUTRITIONAL ASSESSMENT

Group	Patient	Weight/Height (% Standard)	Triceps Skin Fold (% Standard)	Arm Muscle Circumference (% Standard)	Albumin (g/dl)	Transferrin (mg/dl)	Status
A	A.B.	119	192	96	4.3	203	Nourished
	R.B.	217	480	131	4.7	285	Nourished
	M.C.	89	48	88	3.4	164	Moderately Malnourished
	L.C.	101	80	105	4.3	220	Nourished
	M.G.	90	79	90	4.4	240	Nourished
	R.McD.	110	88	91	3.5	260	Nourished
	H.M.	91	76	93	3.5	220	Nourished
B	T.C.	93	96	98	3.8	240	Nourished
	C.E.	91	52	95	4.1	201	Nourished
	R.F.	101	97	80	3.4	162	Moderately Malnourished
	T.F.	168	278	112	4.1	235	Nourished
	A.G.	110	136	89	4.3	265	Slightly Malnourished

(Over)

Group	Patient	Weight/Height (% Standard)	Triceps Skin Fold (% Standard)	Arm Muscle Circumference (% Standard)	Albumin (g/dl)	Transferrin (mg/dl)	Status
B	H.G.	103	56	104	3.9	280	Nourished
	C.La.	100	80	92	4.0	300	Nourished
	C.Li.	91	48	90	4.2	265	Slightly Malnourished
	C.McD.	110	103	102	3.4	132	Moderately Malnourished
C	R.B.	125	152	105	4.3	200	Nourished
	R.G.	128	160	95	4.1	235	Nourished
	J.G.	108	48	98	3.9	275	Slightly Malnourished
	G.H.	146	112	113	5.0	330	Nourished
	G.O'B.	118	---	---	4.6	280	Nourished
	R.R.	87	40	89	3.4	173	Moderately Malnourished
	D.R.	93	48	96	4.4	230	Slightly Malnourished
	M.S.	144	108	106	4.3	280	Nourished

TABLE 5 - ALBUMIN AND PLASMA INFUSIONS

TABLE 5 - ALBUMIN AND PLASMA INFUSIONS

Salt Poor Albumin = SPA
Purified Protein Fraction = PPF 25g in 500 ml

Group	Patient	Infusion	Day
A	R.B.	125g SPA	0-1
	M.G.	25g Protein as PPF	0
	R.McD.	25g Protein as PPF	0
B	T.C.	50g SPA	1
	C.E.	40g Protein as PPF	0
		25g SPA	0
	T.F.	12.5g Protein as PPF	0
	A.G.	25g Protein as PPF	0
		25g Protein as PPF	1
		12.5g Protein as PPF	4
	H.G.	25g Protein as PPF	0
	C.McD.	12.5g Protein as PPF	0
		25g SPA	0
C	R.B.	12.5g Protein as PPF	0
	R.G.	12.5g Protein as PPF	0,1,2,3,4
	G.H.	12.5g Protein as PPF	1
	G.O'B.	25g Protein as PPF	0
		37.5g Protein as PPF	1 and 3
		12.5g Protein as PPF	2 and 4
	R.R.	12.5g Protein as PPF	0

TABLE 6 - NITROGEN BALANCES

TABLE 6 - NITROGEN BALANCES

Daily Nitrogen Balance (g N)

	Group A	Group B	Group C
Day 1	-4.7 ± 3.8	-2.6 ± 3.9	-13.5 ± 7.1
Day 2	-5.8 ± 3.2	-2.0 ± 3.1	-10.1 ± 4.7
Day 3	-7.9 ± 2.7	-1.4 ± 5.0	-11.6 ± 5.8
Day 4	-1.3 ± 9.1	-3.8 ± 4.2	-9.0 ± 2.8
Day 5	-6.6 ± 6.5	-5.1 ± 3.5	-8.4 ± 3.1
Day 6	-4.2 ± 2.6	-5.5 ± 7.3	-8.7 ± 3.0
Day 7	-6.9 ± 2.7	-5.0 ± 4.3	-8.7 ± 2.6

Figures quoted as mean ± standard deviation

Cumulative Nitrogen Balance (g N)

	Group A	Group B	Group C
Day 1	-4.7	-2.6	-13.5
Day 2	-10.5	-4.7	-23.5
Day 3	-18.4	-6.1	-35.1
Day 4	-19.7	-9.9	-44.1
Day 5	-26.3	-15.0	-52.8
Day 6	-30.5	-20.5	-61.5
Day 7	-37.4	-24.7	-70.2

TABLE 7 - SUBSTRATE AND INSULIN PROFILES

TABLE 7 - SUBSTRATE AND INSULIN PROFILES

Glucose (mM)	Group A	Group B	Group C
Preop	4.56 ± 1.29	6.22 ± 2.41	6.40 ± 1.30
Postop	7.06 ± 1.89	9.49 ± 2.56	10.20 ± 3.00
Day 2	4.36 ± 0.83	5.24 ± 0.97	7.32 ± 1.62
Day 4	4.71 ± 0.51	4.63 ± 1.12	5.69 ± 1.17
Day 6/7	4.36 ± 0.52	5.36 ± 0.88	7.25 ± 2.50
Lactate (mM)			
Preop	1.18 ± 0.10	1.84 ± 2.06	2.33 ± 1.19
Postop	1.46 ± 0.33	2.19 ± 1.08	2.86 ± 2.12
Day 2	1.40 ± 0.58	1.33 ± 0.83	1.67 ± 1.35
Day 4	0.75 ± 0.33	1.17 ± 0.42	1.46 ± 1.14
Day 6/7	0.37 ± 0.36	0.88 ± 0.29	0.93 ± 0.29
Free Fatty Acids (meq/l)			
Preop	880 ± 245	414 ± 212	214 ± 92
Postop	897 ± 441	1018 ± 484	934 ± 498
Day 2	526 ± 221	566 ± 251	388 ± 169
Day 4	555 ± 224	495 ± 121	259 ± 194
Day 6/7	650 ± 335	686 ± 201	280 ± 174

(Over)

TABLE 7 Continued

	Group A	Group B	Group C
Ketone Bodies (βhydroxybutyrate + acetoacetate) (mM)			
Preop	1.00 ± 0.91	0.08 ± 0.05	0.10 ± 0.10
Postop	0.75 ± 0.80	0.35 ± 0.36	0.16 ± 0.15
Day 2	0.74 ± 0.83	0.34 ± 0.11	0.07 ± 0.06
Day 4	1.13 ± 0.67	0.88 ± 0.25	0.20 ± 0.19
Day 6/7	1.41 ± 1.00	1.18 ± 0.88	0.10 ± 0.09
Insulin (μ units/ml)			
Preop	16 ± 14	13 ± 7	10 ± 3
Postop	30 ± 22	25 ± 18	33 ± 22
Day 2	16 ± 16	21 ± 7	39 ± 29
Day 4	13 ± 15	12 ± 8	29 ± 14
Day 6/7	7 ± 7	11 ± 7	29 ± 20

Figures quoted are mean ± standard deviation

TABLE 8 - ACUTE PHASE PROTEIN PROFILE

TABLE 8 - ACUTE PHASE PROTEIN PROFILES

	Normal Range (97)	Day	Group A	Group B	Group C
α_1-Acid glycoprotein (mg/dl)	55-140	Preop	97 ± 27	90 ± 66	114 ± 103
		Postop	106 ± 36	86 ± 61	91 ± 69
		Day 2	113 ± 28	124 ± 41	141 ± 43
		Day 4	126 ± 40	147 ± 37	154 ± 33
		Day 6/7	116 ± 45	156 ± 64	150 ± 30
α_1-Antitrypsin (mg/dl)	200-400	Preop	357 ± 100	306 ± 96	305 ± 160
		Postop	374 ± 106	248 ± 122	256 ± 114
		Day 2	389 ± 125	451 ± 35	475 ± 59
		Day 4	457 ± 134	566 ± 92	565 ± 121
		Day 6/7	441 ± 117	502 ± 152	483 ± 42
aptoglobin (mg/dl)	100-200	Preop	193 ± 117	193 ± 106	218 ± 156
		Postop	185 ± 99	140 ± 136	138 ± 116
		Day 2	187 ± 70	182 ± 103	184 ± 121
		Day 4	257 ± 82	270 ± 80	312 ± 90
		Day 6/7	197 ± 80	256 ± 89	340 ± 77
α_2-Macroglobulin (mg/dl)	150-350	Preop	205 ± 32	216 ± 32	207 ± 70
		Postop	218 ± 39	229 ± 46	219 ± 54
		Day 2	206 ± 59	203 ± 51	196 ± 50
		Day 4	213 ± 62	188 ± 33	200 ± 58
		Day 6/7	199 ± 16	214 ± 49	206 ± 49

(Over)

TABLE 8 Continued

	Normal Range	Day	Group A	Group B	Group C
Ceruloplasmin (mg/dl)	15-60	Preop	26 ± 4	23 ± 7	23 ± 7
		Postop	24 ± 3	20 ± 7	20 ± 5
		Day 2	24 ± 4	21 ± 6	19 ± 4
		Day 4	28 ± 5	23 ± 8	22 ± 3
		Day 6/7	25 ± 5	24 ± 4	22 ± 4
C-reactive Protein (mg/dl)	1.2	Preop	6 ± 6	4 ± 6	4 ± 6
		Postop	13 ± 11	4 ± 6	4 ± 6
		Day 2	20 ± 9	22 ± 12	25 ± 8
		Day 4	15 ± 9	19 ± 9	15 ± 7
		Day 6/7	11 ± 11	13 ± 10	9 ± 6
C_3 complement (mg/dl)	100-200	Preop	164 ± 17	135 ± 27	146 ± 70
		Postop	162 ± 13	122 ± 20	122 ± 37
		Day 2	156 ± 29	123 ± 23	137 ± 31
		Day 4	182 ± 31	145 ± 14	165 ± 35
		Day 6/7	177 ± 37	153 ± 21	169 ± 31

Figures quoted are mean ± standard deviation

TABLE 9 - IMMUNOGLOBULIN PROFILES

TABLE 9 - IMMUNOGLOBULIN PROFILES

	Normal Range	Day	Group A	Group B	Group C
IgA (mg/dl)	50-400	Preop	227 ± 129	193 ± 61	192 ± 168
		Postop	225 ± 103	219 ± 71	172 ± 99
		Day 2	192 ± 91	184 ± 75	155 ± 79
		Day 4	219 ± 84	188 ± 76	180 ± 101
		Day 6/7	257 ± 98	296 ± 77	203 ± 113
IgM	60-250	Preop	69 ± 26	107 ± 78	80 ± 107
		Postop	82 ± 17	115 ± 73	71 ± 56
		Day 2	74 ± 34	103 ± 81	70 ± 46
		Day 4	71 ± 29	106 ± 78	79 ± 58
		Day 6/7	80 ± 37	143 ± 113	98 ± 58
IgG	650-1600	Preop	872 ± 278	900 ± 232	924 ± 292
		Postop	784 ± 226	837 ± 146	913 ± 198
		Day 2	760 ± 178	728 ± 224	762 ± 242
		Day 4	832 ± 172	697 ± 200	704 ± 236
		Day 6/7	846 ± 249	861 ± 281	776 ± 308

Figures quoted are mean ± standard deviation

TABLE 10 - NUTRITIONAL MARKER PROFILES

TABLE 10 - NUTRITIONAL MARKERS

arker	Normal Range	Day	Group A	Group B	Group C
lbumin g/dl)	3.5 - 5.5	Preop	4.1 ± 0.9	3.9 ± 0.8	3.9 ± 0.9
		Postop	4.3 ± 0.8	3.6 ± 0.7	4.1 ± 0.8
		Day 2	3.7 ± 1.0	3.2 ± 0.5	3.4 ± 0.7
		Day 4	3.8 ± 0.8	3.0 ± 0.5	3.1 ± 0.5
		Day 6/7	3.8 ± 0.8	3.1 ± 0.5	2.9 ± 0.2
ransferrin mg/dl)	200 - 400	Preop	249 ± 39	244 ± 33	262 ± 70
		Postop	242 ± 54	223 ± 45	242 ± 57
		Day 2	235 ± 74	190 ± 53	212 ± 43
		Day 4	231 ± 79	181 ± 48	199 ± 31
		Day 6/7	200 ± 50	175 ± 52	234 ± 62
realbumin mg/dl)	10 - 40	Preop	16.0 ± 7.1	15.8 ± 7.9	20.8 ± 5.2
		Postop	15.3 ± 7.3	18.0 ± 6.6	20.5 ± 6.3
		Day 2	10.8 ± 4.0	8.5 ± 5.3	11.3 ± 4.0
		Day 4	10.5 ± 5.1	5.9 ± 7.3	9.9 ± 2.8
		Day 6/7	9.8 ± 4.9	6.7 ± 2.0	11.4 ± 2.5

igures quoted are mean ± standard deviation

TABLE 11 - DAYS OF FEVER AND INFECTIONS

TABLE 11 - DAYS OF FEVER AND INFECTIONS

yrexia of 100°F or greater

Group A	Group B	Group C
.6 days/patient	2.4 days/patient	4.0 days/patient

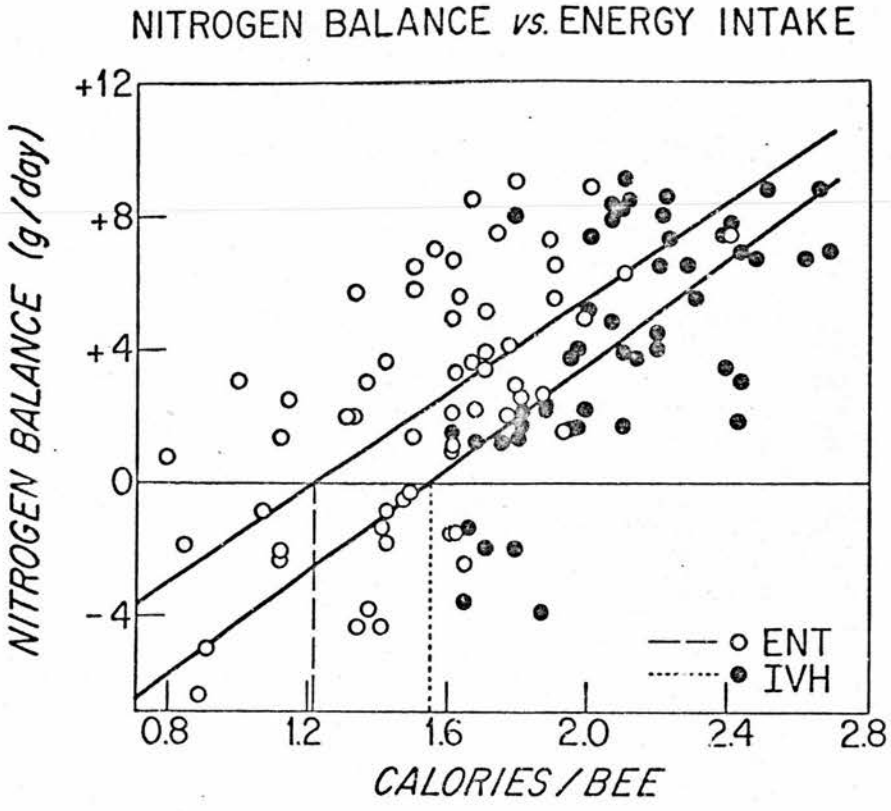
nfections

Group A		Group B		Group C	
ound Infection	1	Wound Infection	1	Urinary Tract Infection	1
		Pyocystitis	1	Chest Infection	1
				X-ray atelectasis - No positive culture	2

FIGURES

FIGURE 1 - NITROGEN BALANCE vs ENERGY INTAKE

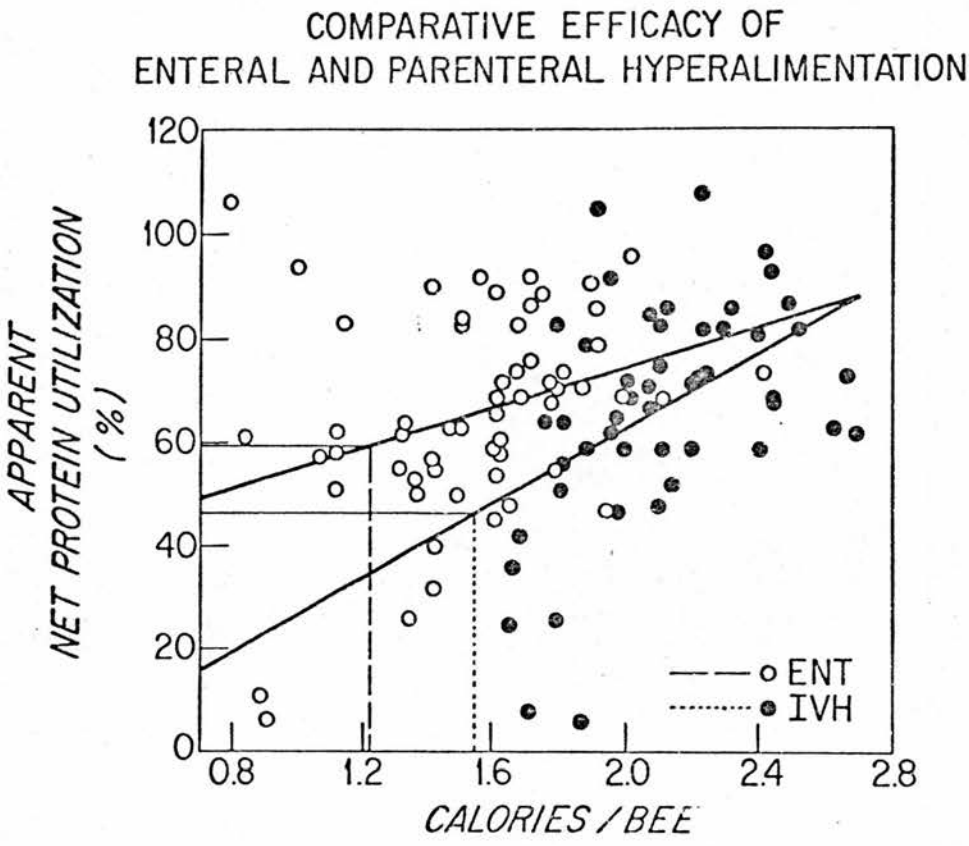
FIGURE 1



The upper line is a linear regression of Nitrogen Balance vs. Calorie/BEE ratio for enteral feeding ($y = 7.13x - 8.68$; $r = 0.62$, p 0.001). The lower line is a similar regression for IVH ($y = 7.83x - 12.09$; $r = 0.64$, p 0.001). Nitrogen equilibrium was achieved with a caloric intake of 1.21 x BEE for enteral nutrition and 1.55 x BEE for IVH.

FIGURE 2 - COMPARATIVE EFFICACY OF ENTERAL AND PARENTERAL HYPERALIMENTATION

FIGURE 2



The upper line is a linear regression of Apparent Net Protein Utilization vs. Calorie/BEE ratio for enteral feeding ($y = 0.20x + 0.36$; $r = 0.32$, p 0.015). The lower line is a similar regression for IVH ($y = 0.36x - 0.09$; $r = 0.47$, p 0.001). At the caloric intake to produce nitrogen equilibrium for enteral nutrition (1.21 x BEE) the NPU was 60%, but at the higher intake with IVH (1.55 x BEE) the NPU was only 47%.

FIGURE 3 - NITROGEN INTAKE

STUDY II

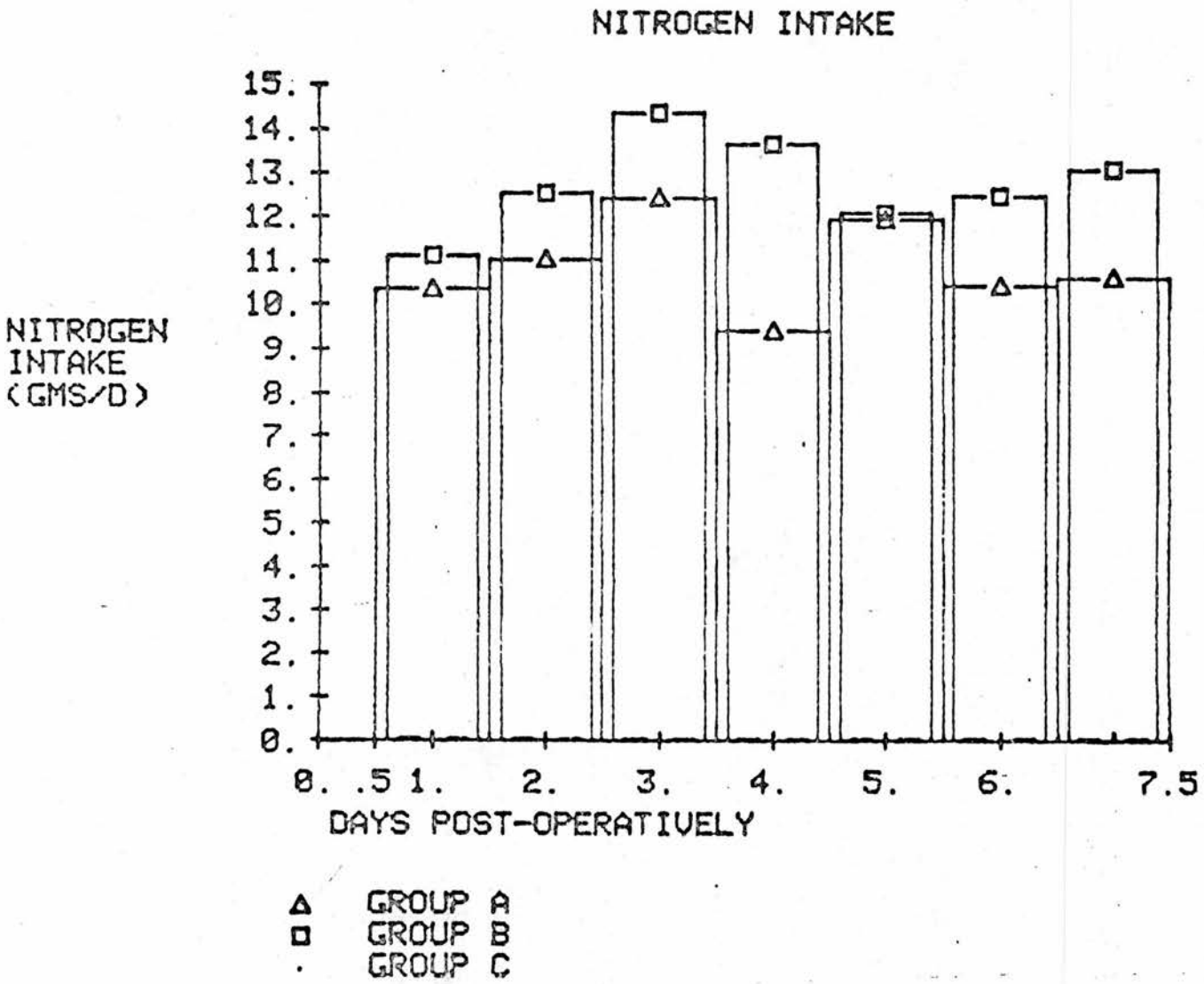


FIGURE 3

FIGURE 4 - NITROGEN BALANCE

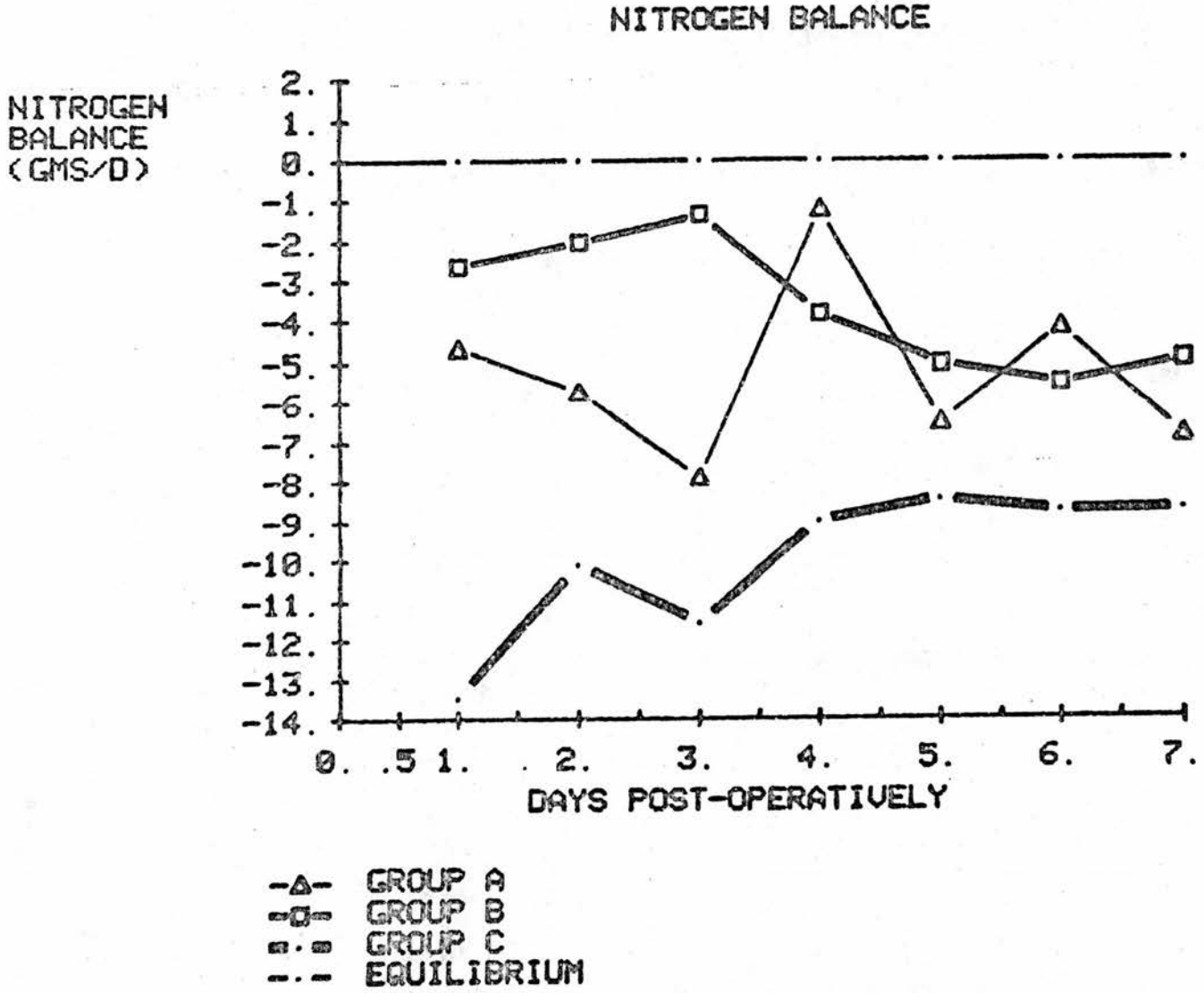

NITROGEN BALANCE
NITROGEN BALANCE (GMS/D)
2.
1.
0.
-1.
-2.
-3.
-4.
-5.
-6.
-7.
-8.
-9.
-10.
-11.
-12.
-13.
-14.
0. .5 1. 2. 3. 4. 5. 6. 7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C
EQUILIBRIUM

FIGURE 4

FIGURE 5 - BLOOD GLUCOSE PROFILE

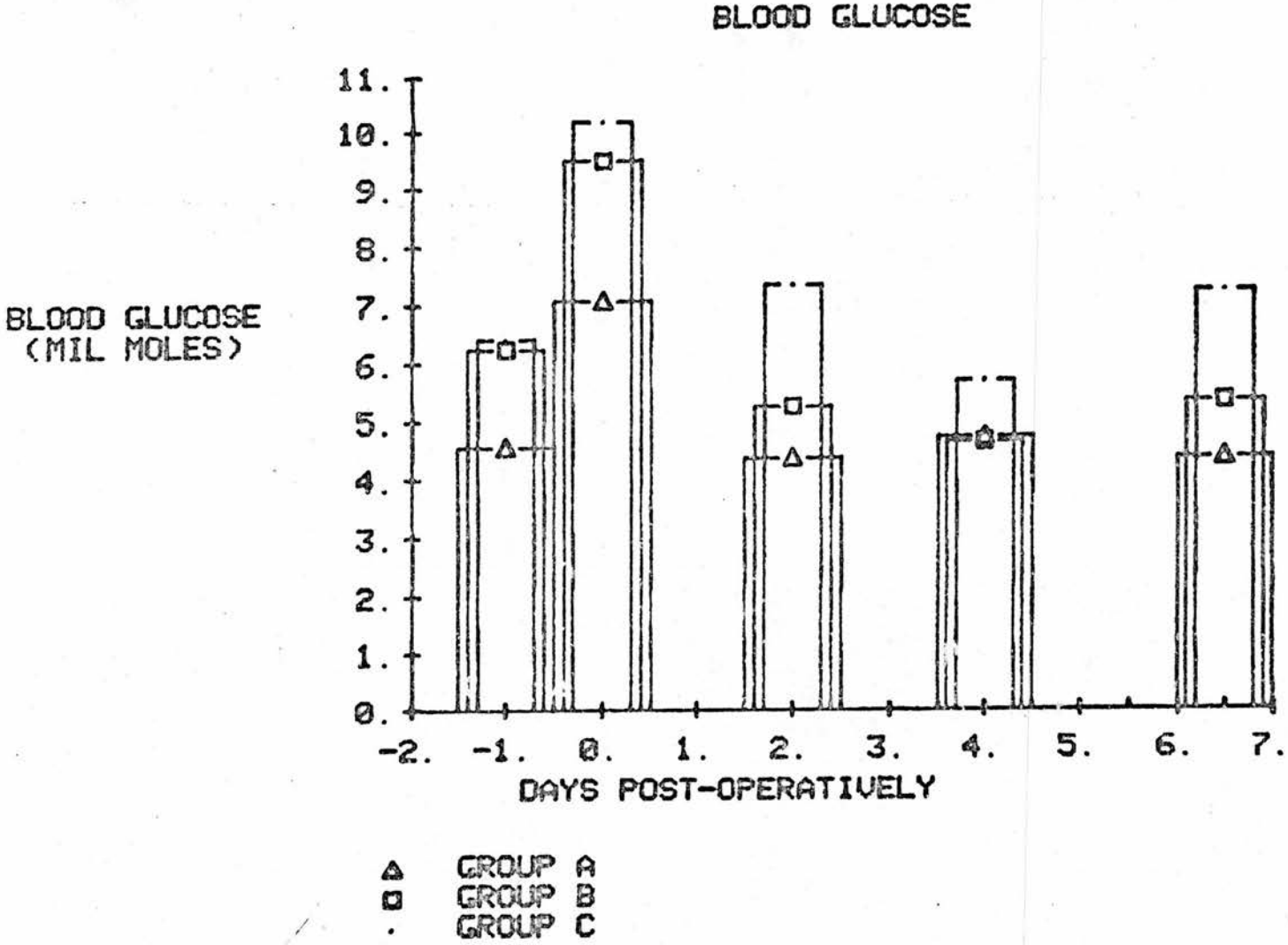

BLOOD GLUCOSE
BLOOD GLUCOSE
(MIL MOLES)
11.
10.
9.
8.
7.
6.
5.
4.
3.
2.
1.
0.
-2.
-1.
0.
1.
2.
3.
4.
5.
6.
7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 5

FIGURE 6 - BLOOD LACTATE PROFILE

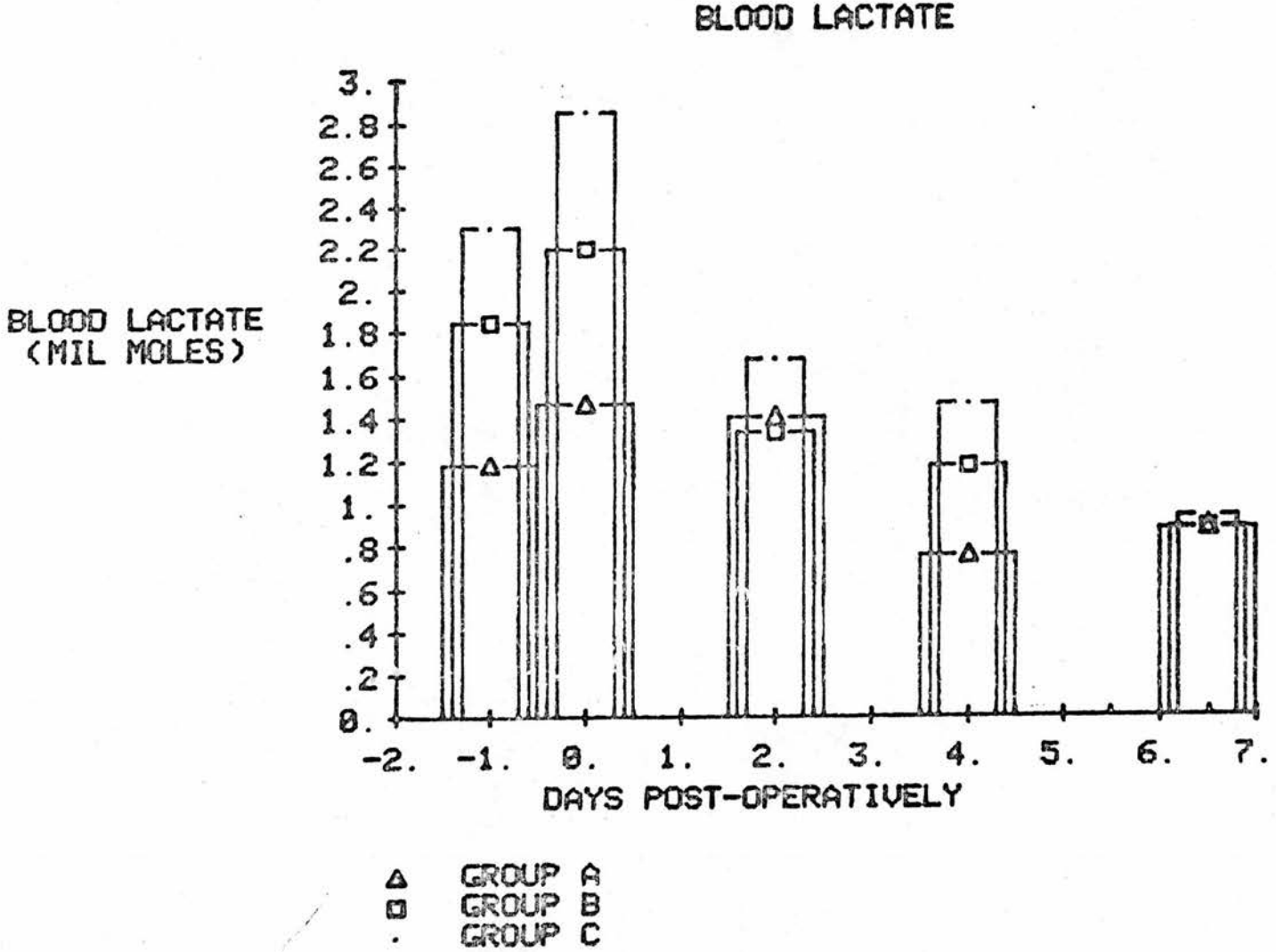

BLOOD LACTATE
BLOOD LACTATE
(MIL MOLES)
3.
2.8
2.6
2.4
2.2
2.
1.8
1.6
1.4
1.2
1.
.8
.6
.4
.2
0.
-2.
-1.
0.
1.
2.
3.
4.
5.
6.
7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 6

FIGURE 7 - PLASMA FREE FATTY ACID PROFILE

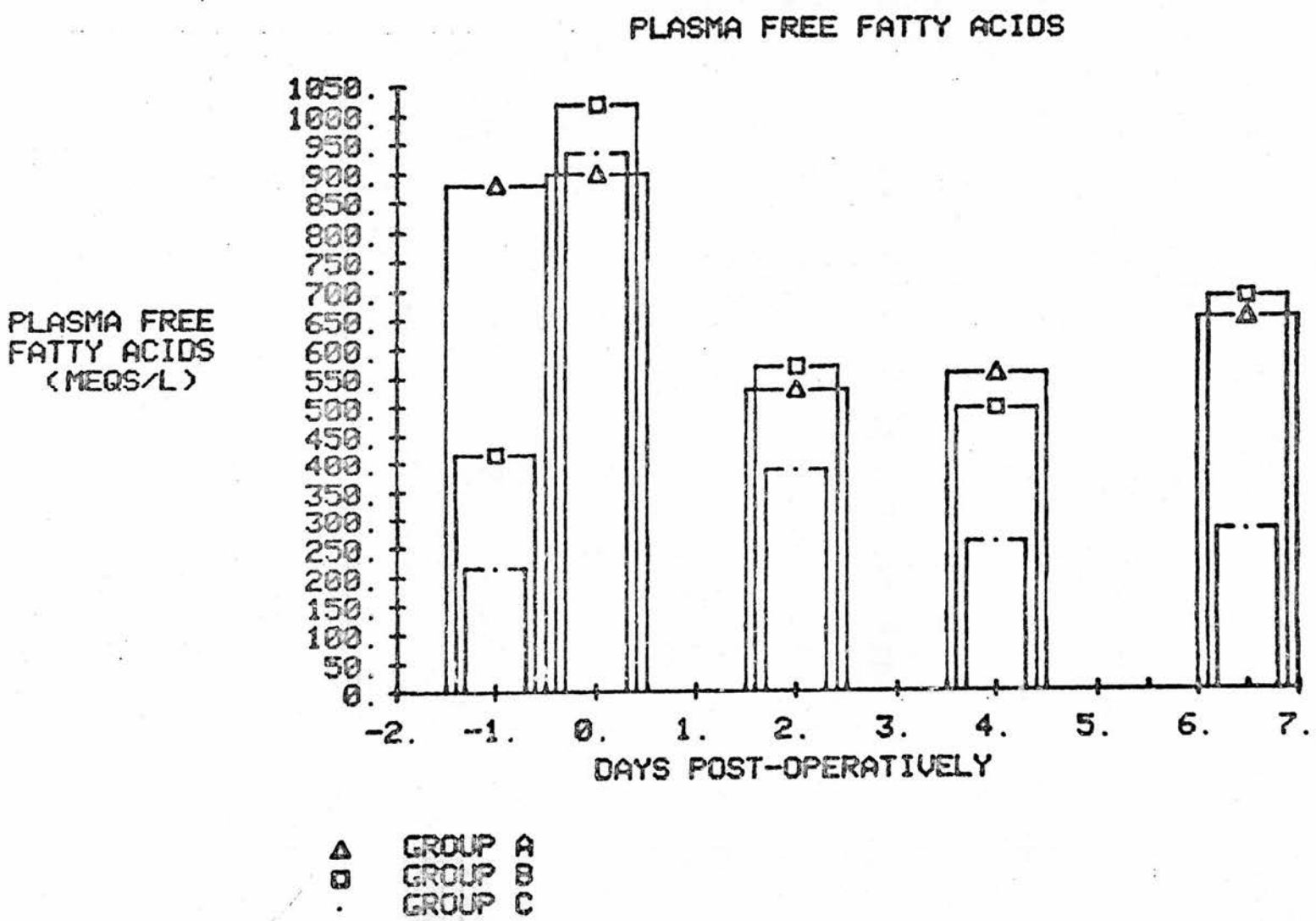

PLASMA FREE FATTY ACIDS
PLASMA FREE FATTY ACIDS (MEQS/L)
1050.
1000.
950.
900.
850.
800.
750.
700.
650.
600.
550.
500.
450.
400.
350.
300.
250.
200.
150.
100.
50.
0.
-2. -1. 0. 1. 2. 3. 4. 5. 6. 7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 7

FIGURE 8 - TOTAL BLOOD KETONE BODY PROFILE

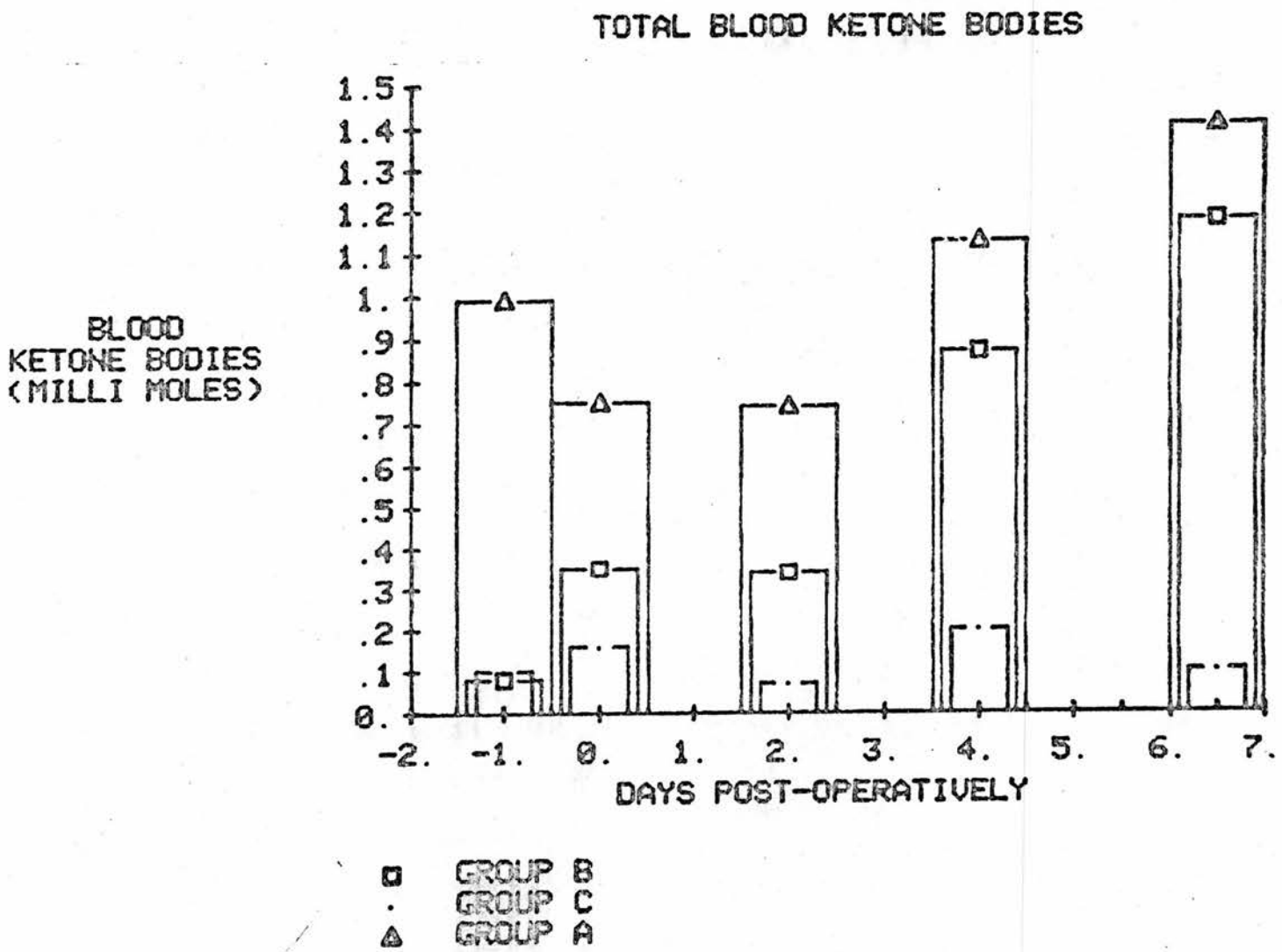

TOTAL BLOOD KETONE BODIES
BLOOD
KETONE BODIES
(MILLI MOLES)
1.5
1.4
1.3
1.2
1.1
1.
.9
.8
.7
.6
.5
.4
.3
.2
.1
0.
-2.
-1.
0.
1.
2.
3.
4.
5.
6.
7.
DAYS POST-OPERATIVELY
GROUP B
GROUP C
GROUP A

FIGURE 8

FIGURE 9 - SERUM INSULIN PROFILE

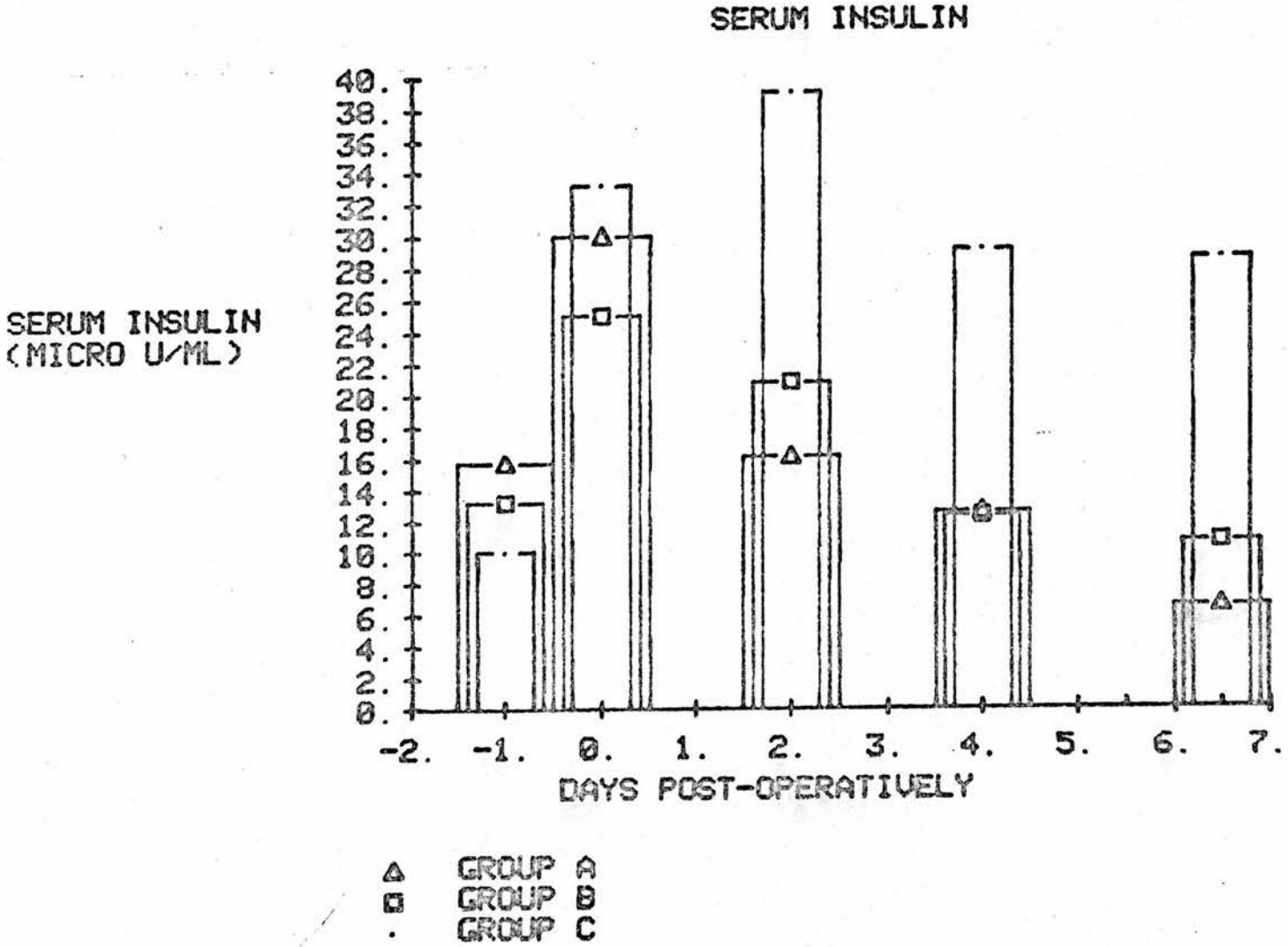

SERUM INSULIN
SERUM INSULIN
(MICRO U/ML)
40.
38.
36.
34.
32.
30.
28.
26.
24.
22.
20.
18.
16.
14.
12.
10.
8.
6.
4.
2.
0.
-2.
-1.
0.
1.
2.
3.
4.
5.
6.
7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 9

FIGURE 10 - α_1-ACID GLYCOPROTEIN PROFILE

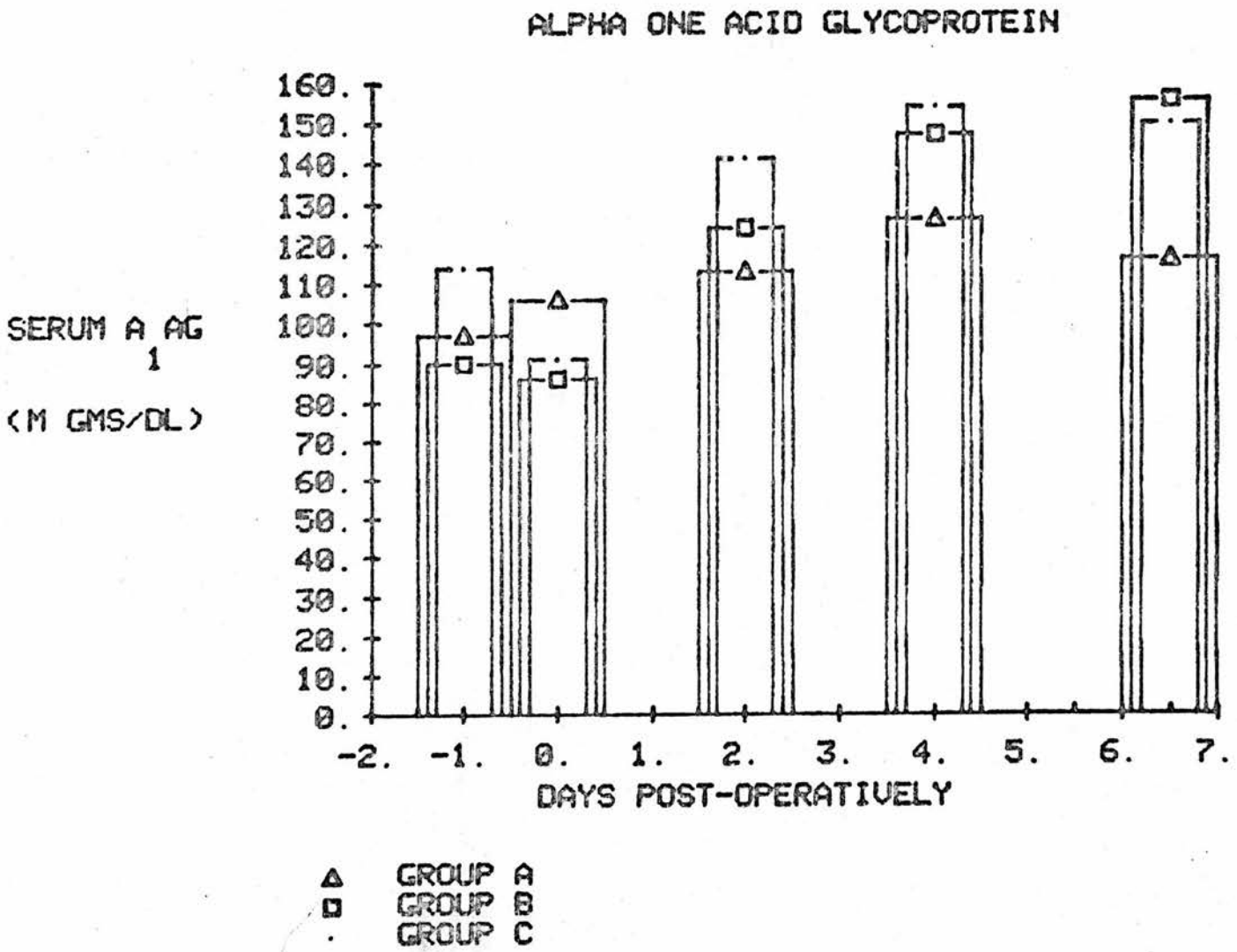

ALPHA ONE ACID GLYCOPROTEIN
SERUM A AG 1
(M GMS/DL)
160.
150.
140.
130.
120.
110.
100.
90.
80.
70.
60.
50.
40.
30.
20.
10.
0.
-2. -1. 0. 1. 2. 3. 4. 5. 6. 7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 1o

FIGURE 11 - α_1-ANTI-TRYPSIN PROFILE

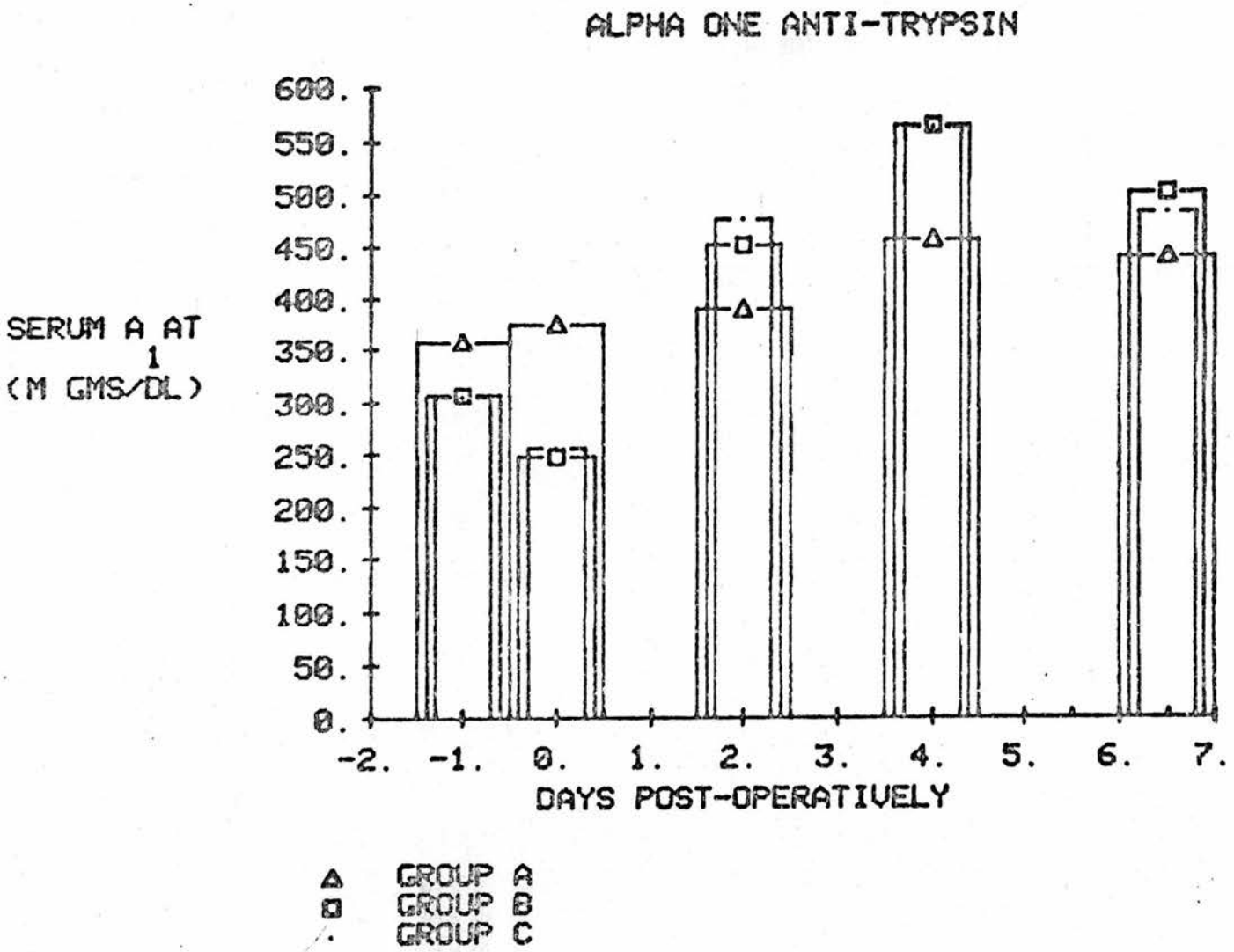

ALPHA ONE ANTI-TRYPSIN
SERUM A AT 1 (M GMS/DL)
600.
550.
500.
450.
400.
350.
300.
250.
200.
150.
100.
50.
0.
-2. -1. 0. 1. 2. 3. 4. 5. 6. 7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 11

FIGURE 12 - HAPTOGLOBIN PROFILE

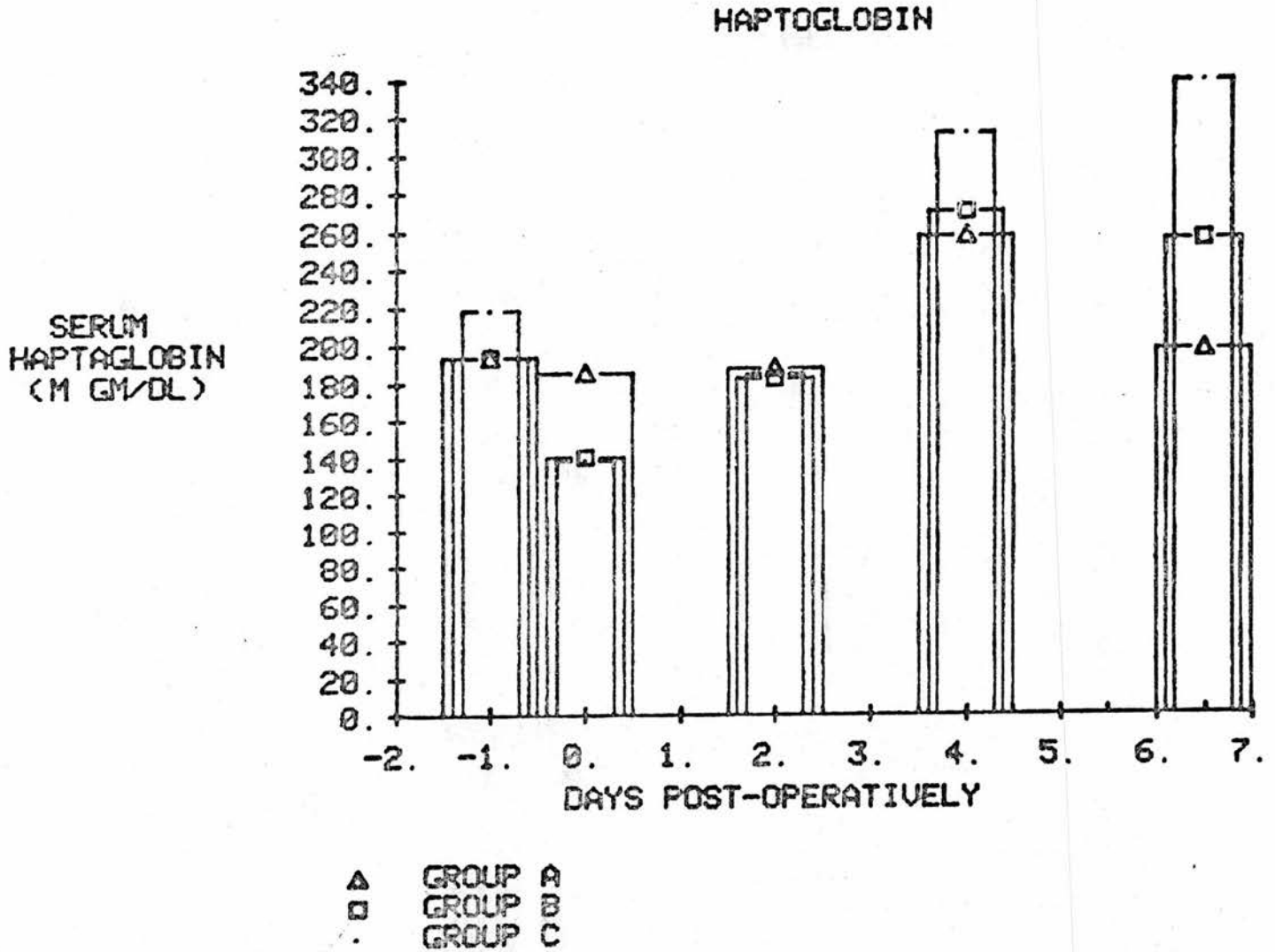

HAPTOGLOBIN
SERUM HAPTAGLOBIN (M GM/DL)
340.
320.
300.
280.
260.
240.
220.
200.
180.
160.
140.
120.
100.
80.
60.
40.
20.
0.
-2. -1. 0. 1. 2. 3. 4. 5. 6. 7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 12

FIGURE 13 - C_3 COMPLEMENT PROFILE

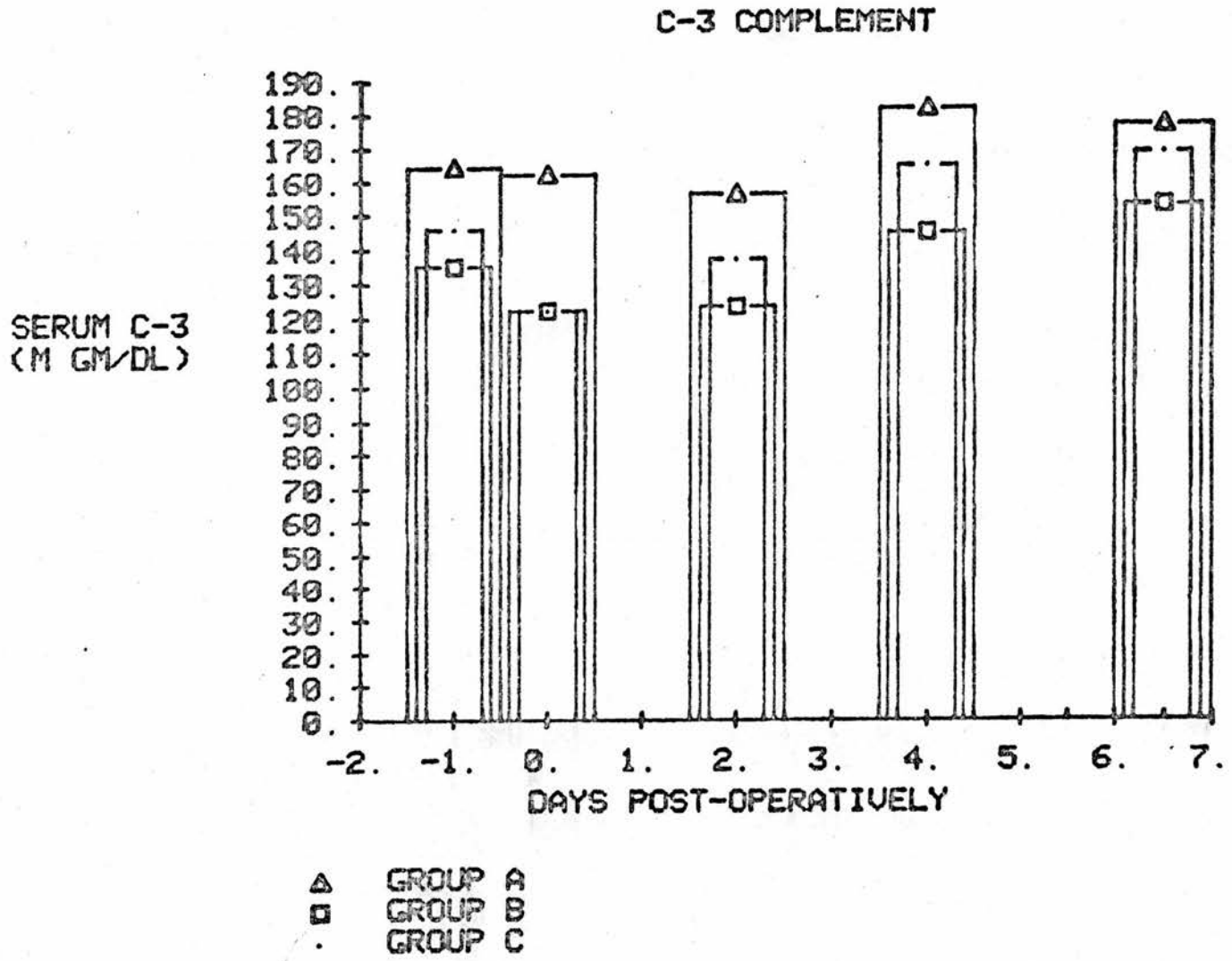

C-3 COMPLEMENT
SERUM C-3
(M GM/DL)
190.
180.
170.
160.
150.
140.
130.
120.
110.
100.
90.
80.
70.
60.
50.
40.
30.
20.
10.
0.
-2. -1. 0. 1. 2. 3. 4. 5. 6. 7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 13

FIGURE 14 - C REACTIVE PROTEIN PROFILE

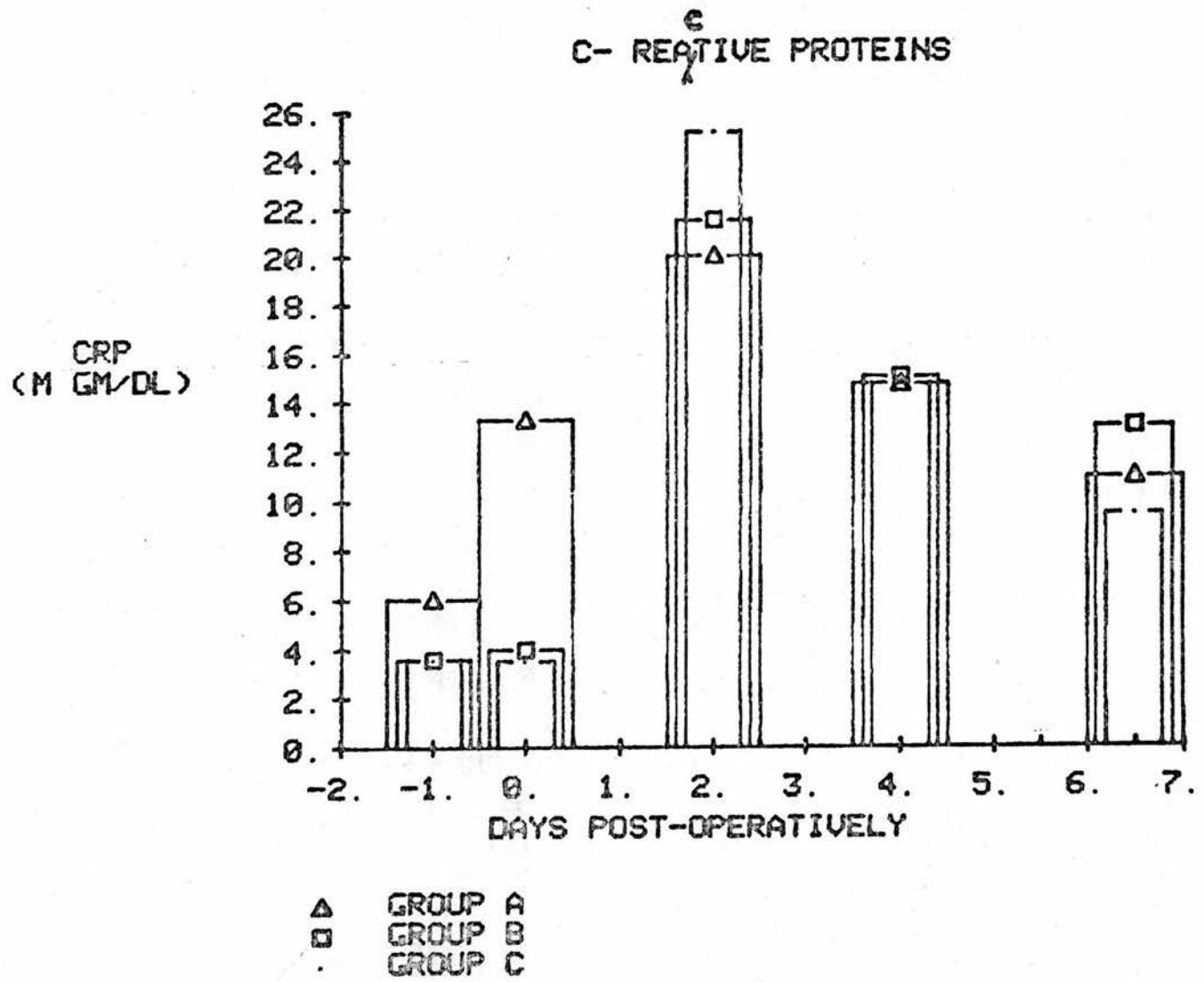

C- REACTIVE PROTEINS
CRP
(M GM/DL)
26.
24.
22.
20.
18.
16.
14.
12.
10.
8.
6.
4.
2.
0.
-2. -1. 0. 1. 2. 3. 4. 5. 6. 7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 14

FIGURE 15 - α_2 MACROGLOBULIN PROFILE

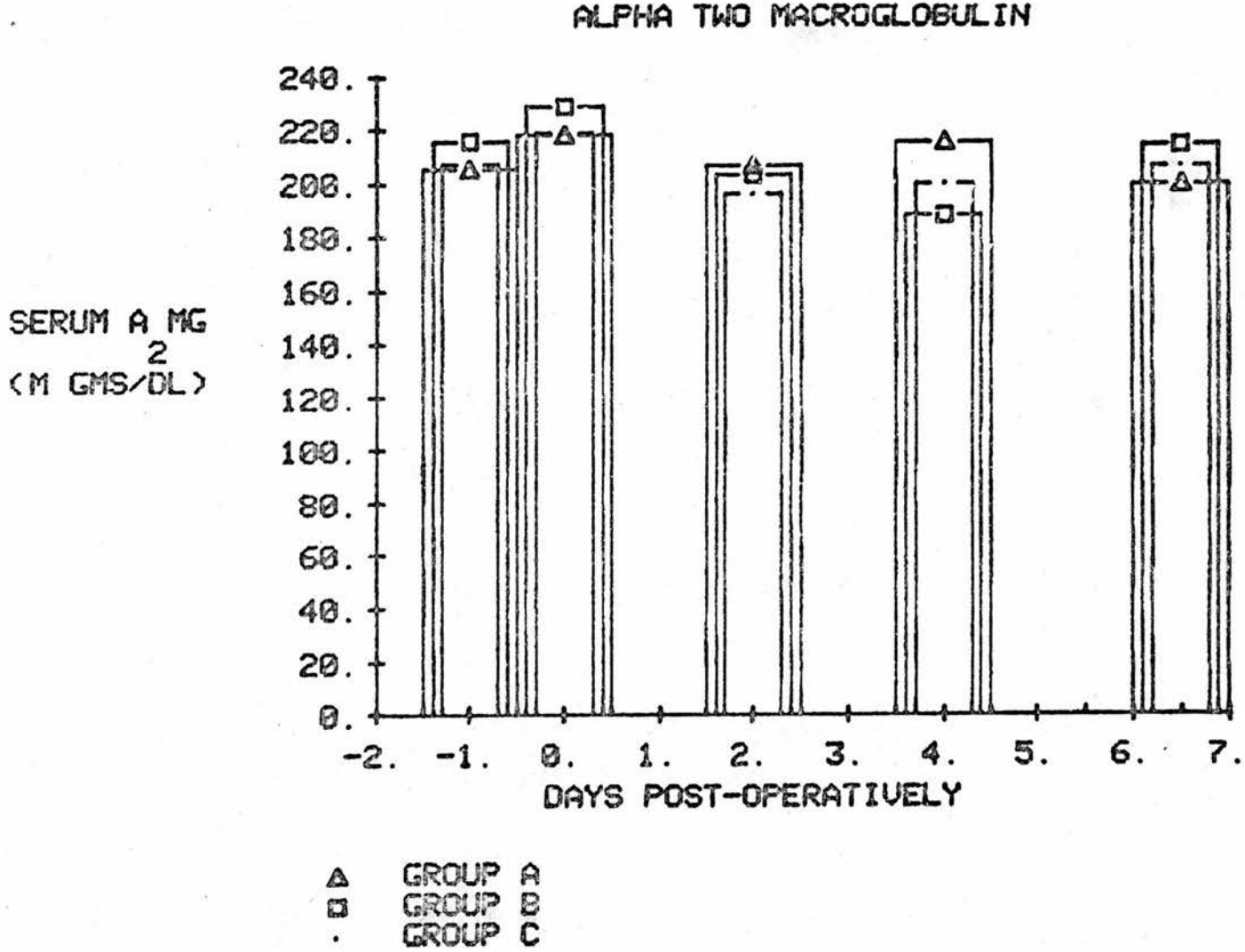

ALPHA TWO MACROGLOBULIN
SERUM A MG
2
(M GMS/DL)
240.
220.
200.
180.
160.
140.
120.
100.
80.
60.
40.
20.
0.
-2.
-1.
0.
1.
2.
3.
4.
5.
6.
7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 15

FIGURE 16 - CERULOPLASMIN PROFILE

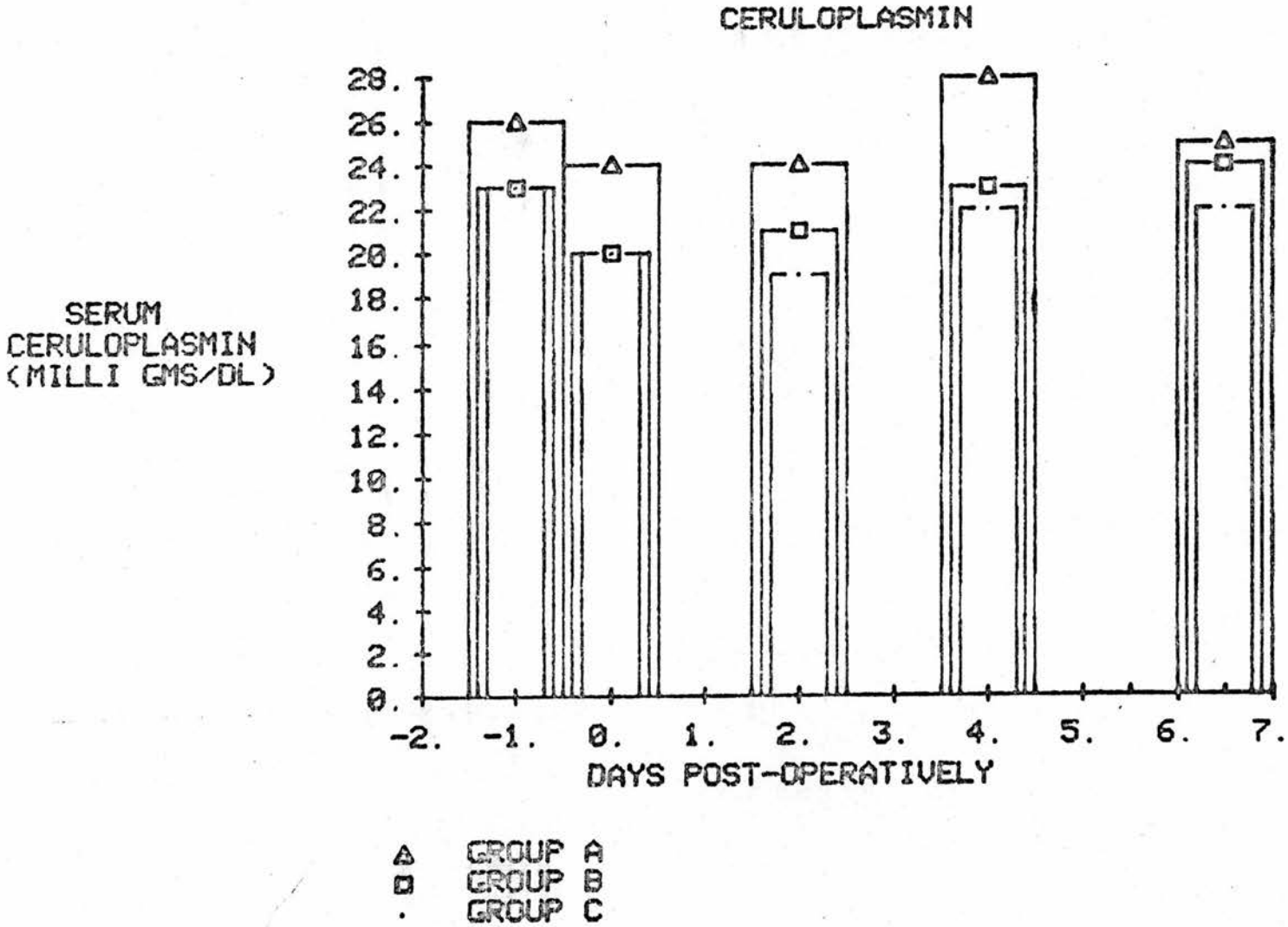

CERULOPLASMIN
SERUM
CERULOPLASMIN
(MILLI GMS/DL)
28.
26.
24.
22.
20.
18.
16.
14.
12.
10.
8.
6.
4.
2.
0.
-2.
-1.
0.
1.
2.
3.
4.
5.
6.
7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 16

FIGURE 17 - IMMUNOGLOBULIN A PROFILE

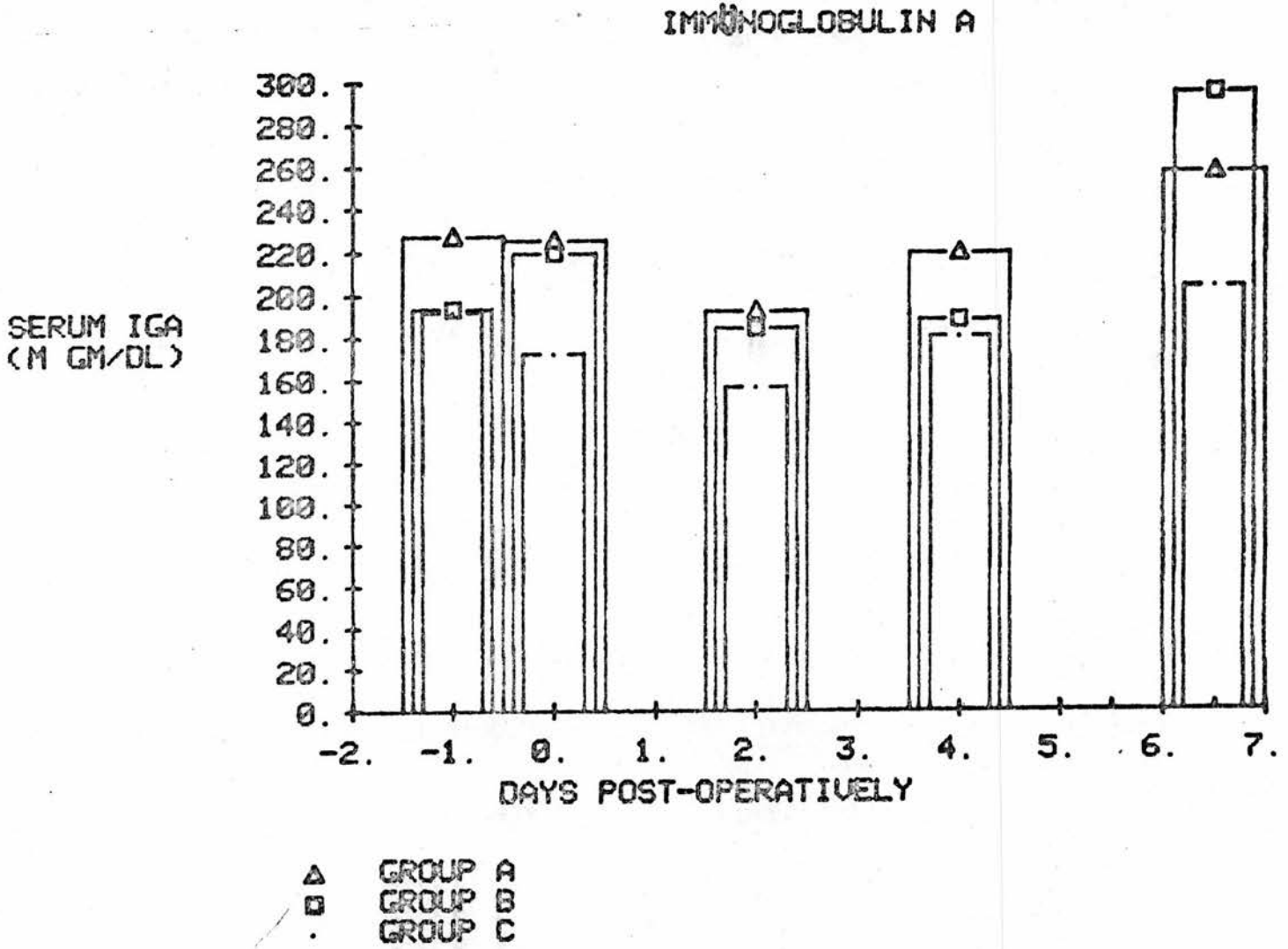

IMMUNOGLOBULIN A
SERUM IGA
(M GM/DL)
300.
280.
260.
240.
220.
200.
180.
160.
140.
120.
100.
80.
60.
40.
20.
0.
-2.
-1.
0.
1.
2.
3.
4.
5.
6.
7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 17

FIGURE 18 - IMMUNOGLOBULIN M PROFILE

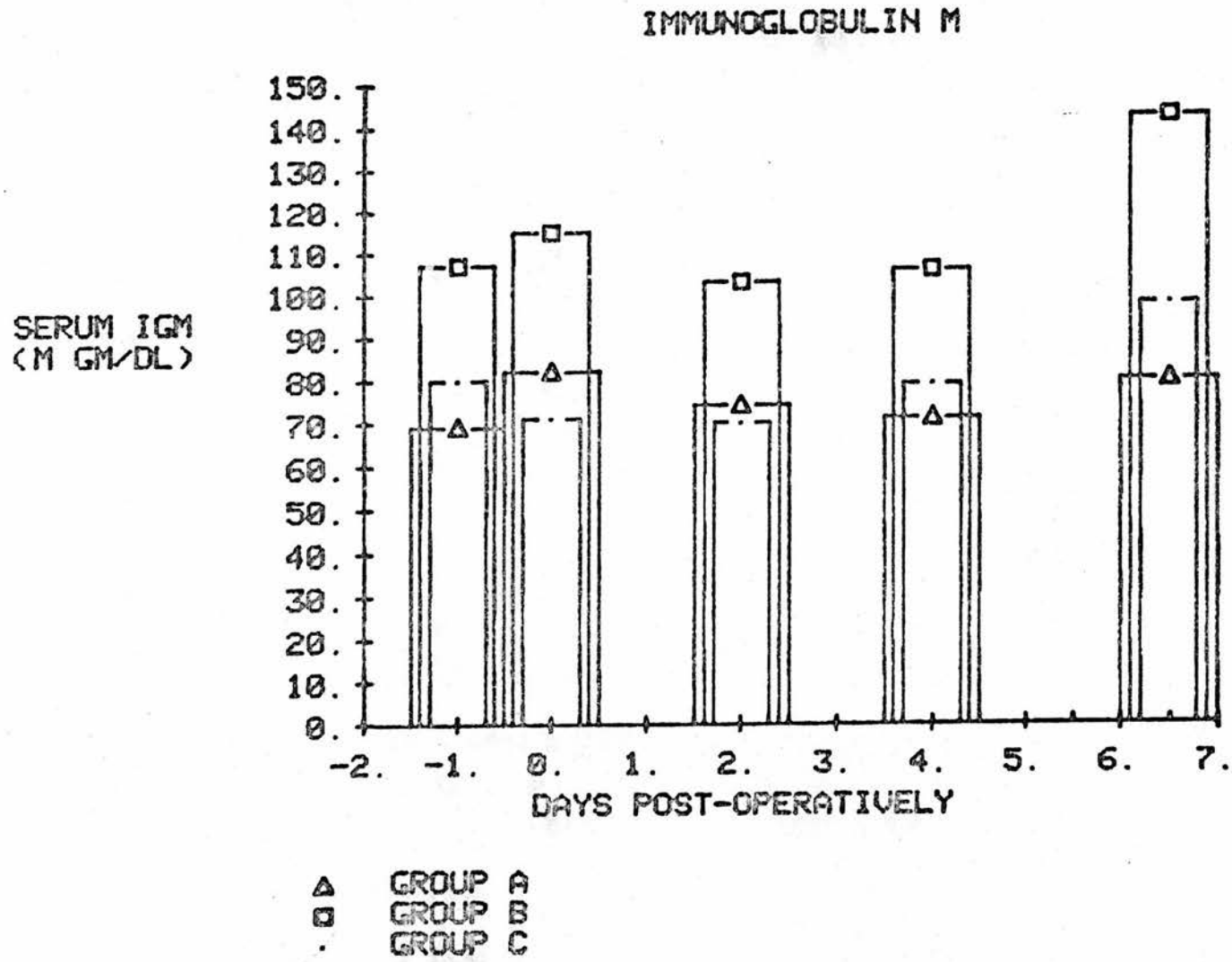

IMMUNOGLOBULIN M
SERUM IGM
(M GM/DL)
150.
140.
130.
120.
110.
100.
90.
80.
70.
60.
50.
40.
30.
20.
10.
0.
-2. -1. 0. 1. 2. 3. 4. 5. 6. 7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 18

FIGURE 19 - IMMUNOGLOBULIN G PROFILE

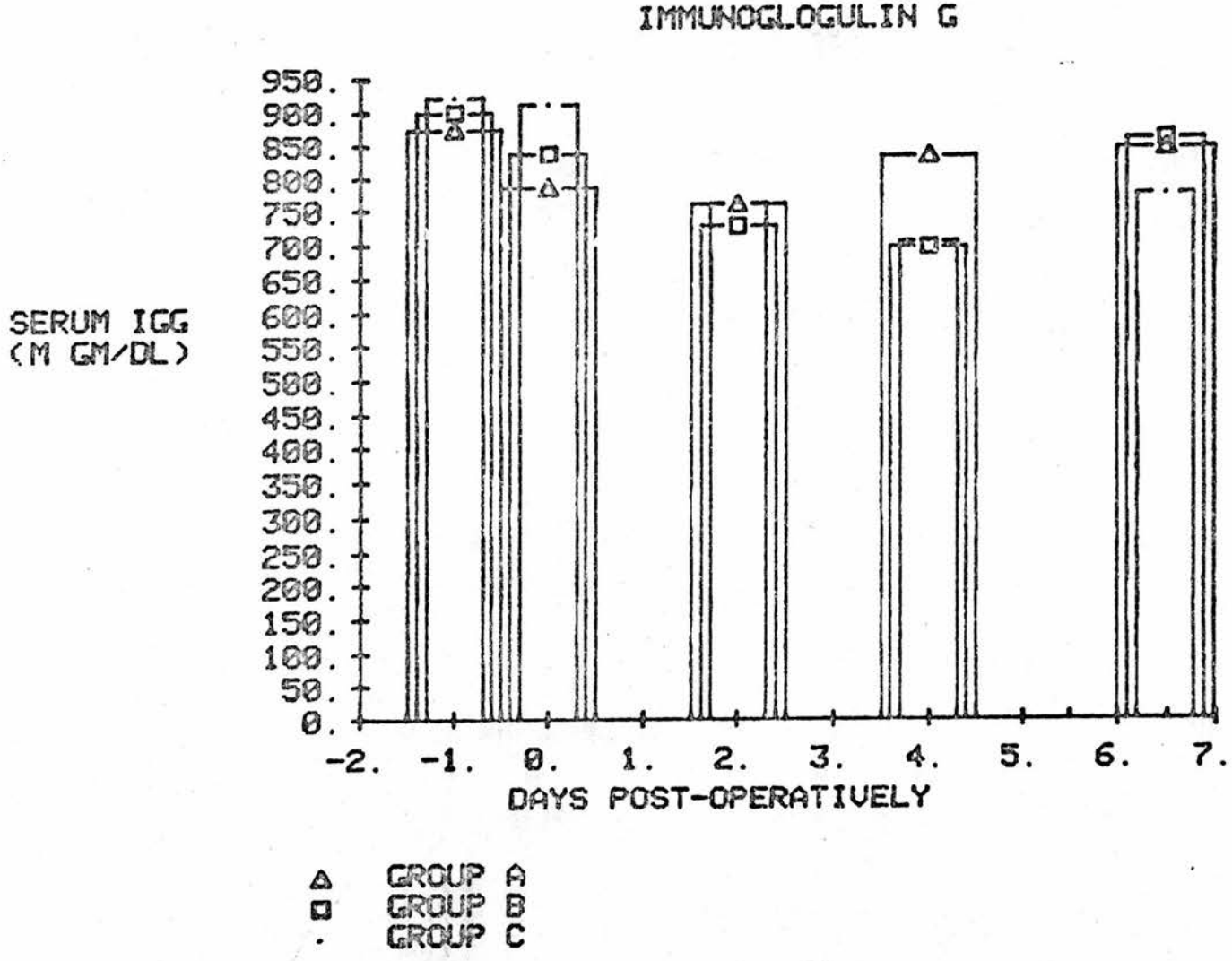

IMMUNOGLOGULIN G
SERUM IGG
(M GM/DL)
950.
900.
850.
800.
750.
700.
650.
600.
550.
500.
450.
400.
350.
300.
250.
200.
150.
100.
50.
0.
-2. -1. 0. 1. 2. 3. 4. 5. 6. 7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 19

FIGURE 20 - ALBUMIN PROFILE

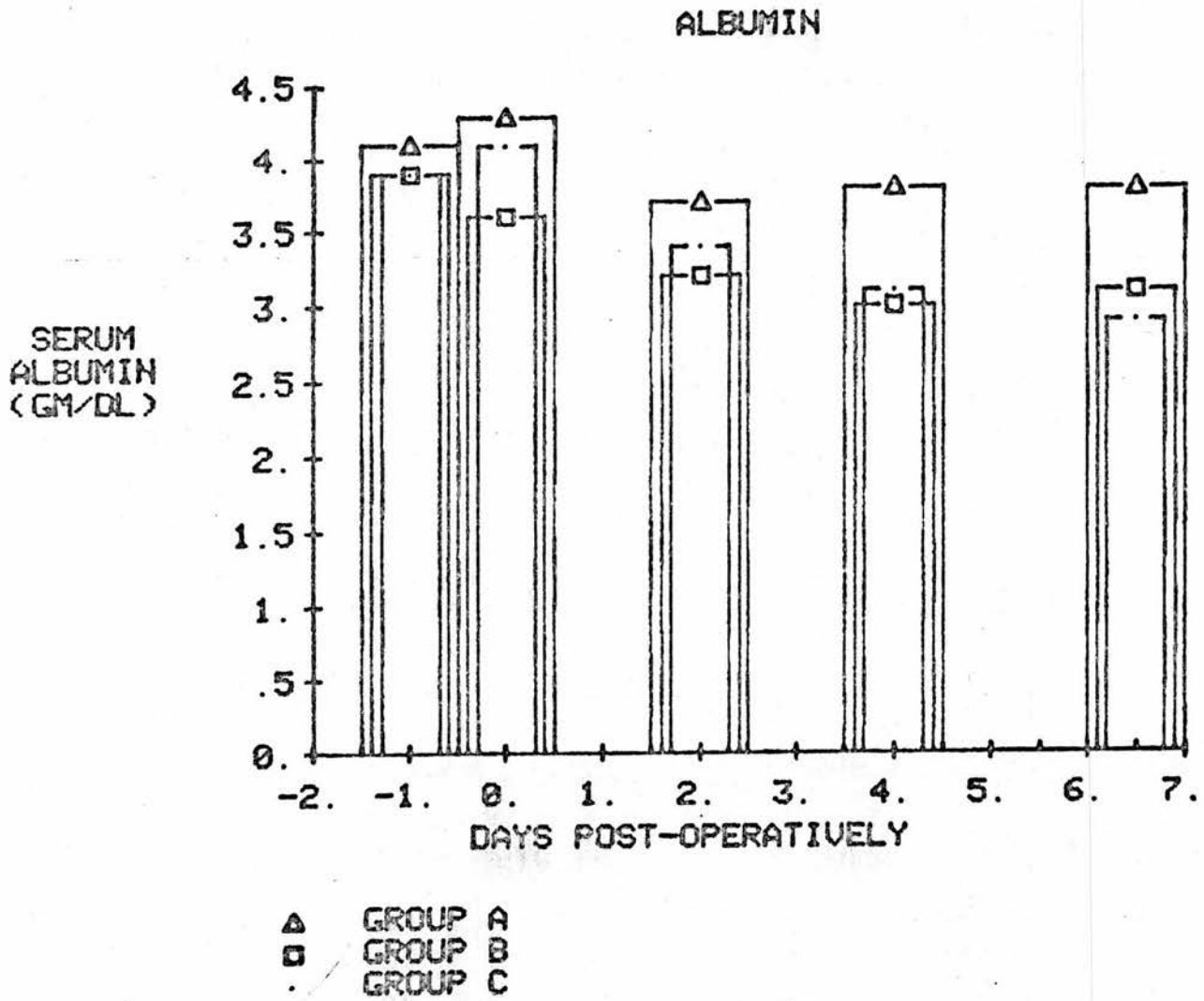

ALBUMIN
SERUM ALBUMIN (GM/DL)
4.5
4.
3.5
3.
2.5
2.
1.5
1.
.5
0.
-2. -1. 0. 1. 2. 3. 4. 5. 6. 7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 20

FIGURE 21 - TRANSFERRIN PROFILE

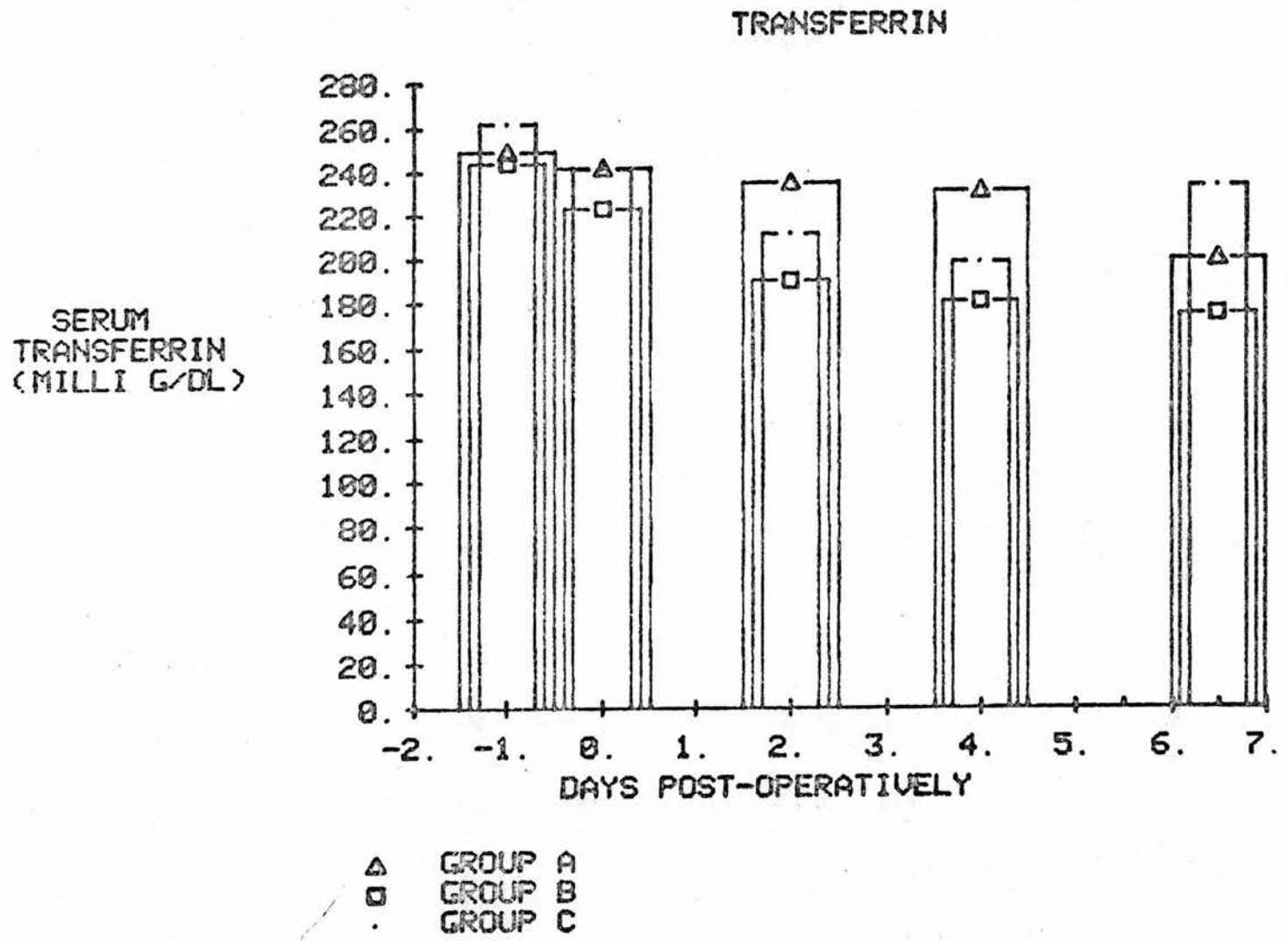

TRANSFERRIN
SERUM
TRANSFERRIN
(MILLI G/DL)
280.
260.
240.
220.
200.
180.
160.
140.
120.
100.
80.
60.
40.
20.
0.
-2.
-1.
0.
1.
2.
3.
4.
5.
6.
7.
DAYS POST-OPERATIVELY
GROUP A
GROUP B
GROUP C

FIGURE 21

FIGURE 22 - PREALBUMIN PROFILE

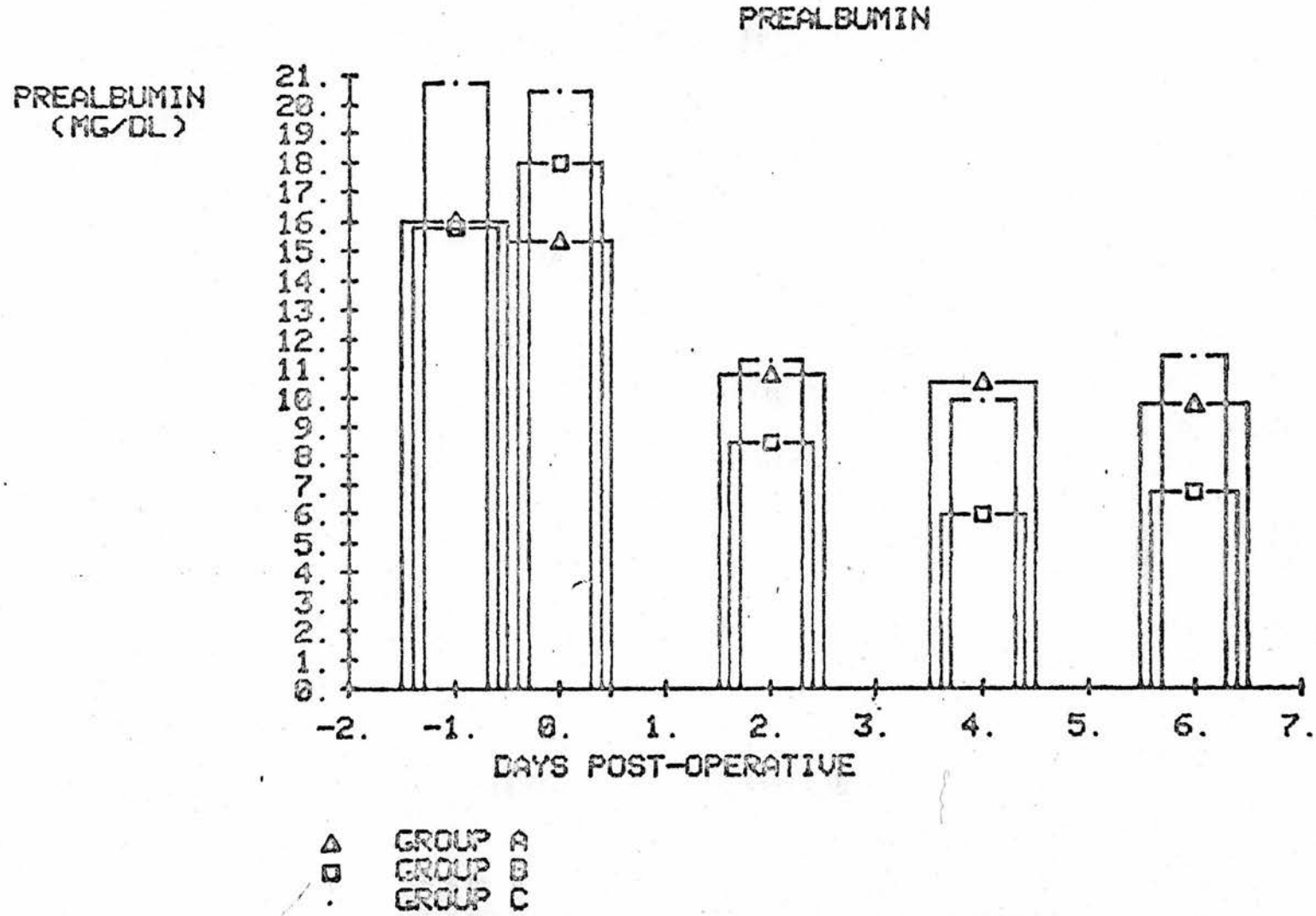

PREALBUMIN
PREALBUMIN
(MG/DL)
21.
20.
19.
18.
17.
16.
15.
14.
13.
12.
11.
10.
9.
8.
7.
6.
5.
4.
3.
2.
1.
0.
-2.
-1.
0.
1.
2.
3.
4.
5.
6.
7.
DAYS POST-OPERATIVE
GROUP A
GROUP B
GROUP C

FIGURE 22